NUTRITIONAL STRATEGIES TO MITIGATE WOODY BREAST AND WHITE STRIPING

A Thesis

by

BROOKE COLETTE BODLE

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Christine Alvarado
Co-Chair of Committee,	Jason Lee
Committee Member,	Stephen Smith
Head of Department,	David Caldwell

December 2017

Major Subject: Poultry Science

ABSTRACT

The aim of this research program was to evaluate the effect of multiple nutritional strategies on broiler performance, meat yield, and the presence and severity of white striping and woody breast in high-yielding, male broilers.

Nutritional strategies explored in Experiment I included an increased level of dArg:dLys, inclusion of vitamin C, doubling the recommended vitamin premix inclusion, or reducing amino acid density 15% during the grower phase. The reduction in amino acid density during Experiment I suppressed body weight and increased feed conversion ratio during the grower and finisher phases. No differences were observed in average breast weight; however, breast yield (as a % of live weight) was significantly reduced in diets containing vitamin C, while all other diets showed similar results to that of the control. Diets containing a higher dArg:dLys, higher vitamin C, and a lower amino acid density significantly reduced the severity of woody breast compared to the industry standard diet. However, no differences in incidence of white striping were observed.

Supplementation of 136 dArg:dLys and 100 ppm vitamin C in Experiment II significantly improved feed consumption and feed conversion ratio. At the termination of the trial, the inclusion of 136 dArg:dLys significantly improved feed conversion ratio compared to the control. No differences in breast weight were observed, however, the diets containing 124 dArg: dLys significantly reduced breast yield compared to the control. Similarly, the diets containing 124 dArg:dLys significantly reduced the severity

of woody breast and white striping compared to the control diet. Furthermore, the control significantly increased the number of severe woody breast scores, compared to diets containing 124 dArg:dLys by 11% and 14.30%, respectively. Similarly, the diets containing 124 dArg:dLys significantly improved the normal and mild white striping scores compared to the positive control by 20.72% and 18.92%, respectively. The inclusion of arginine and vitamin C reduced the severity and incidence of woody breast and white striping compared to an industry standard diet.

DEDICATION

This thesis is dedicated to my family and all of those who have helped shape me into who I am today. Without the overwhelming support, love, and encouragement I received, I would not have been able to achieve this accomplishment.

To my parents, thank you for never ending love and overwhelming support. This journey would have not been possible without you. I am blessed to have two role models that I look up to and strive to be like on a daily basis. The advice and encouragement I received from you, has helped guide me onto a path of success and opportunity.

ACKNOWLEDGEMENTS

I would like to thank the chair and co-chair of my committee Dr. Christine Alvarado and Dr. Jason Lee for providing me with this incredible opportunity. The knowledge I have learned, the experiences I have been a part of, and the friendships I have gained are irreplaceable. None of this would have been possible without your guidance. I consider myself extremely lucky to have had the privilege to work in two of the top poultry laboratories. To my other committee member, Dr. Smith, thank you very much for your guidance and support throughout the course of this research program. You challenged me in each of your classes and I was able to apply that knowledge into my research. I am very thankful for your participation.

I would also like to thank all of the students, both undergraduate and graduate, who have been a part of my career as a Master's student. Without the help of each one of you, I would not have accomplished half of what I did. You have been some of the best co-workers and I am lucky enough to call you some of my best friends. I look forward to seeing where our live take us professionally as we continue to grow. Keep up the hard work and continue to make the laboratories some of the best in the country.

CONTRIBUTORS AND FUNDING SOURCES

This work was supported by a thesis committee consisting of Professor Christine Alvarado (advisor), and Professor Jason Lee (co-advisor) of the Department of Poultry Science, and Professor Stephen Smith of the Department of Animal Science.

The data analyzed for Chapter II and III was provided by Professor Jason Lee. All other work conducted for this thesis was completed by the student independently.

Graduate study was supported by a fellowship from Texas A&M University and funding for this research was made possible by Adisseo – USA.

NOMENCLATURE

BW	Body Weight
d	Day
DDGS	Dried Distillers Grain
FCR	Feed Conversion Ratio
FC	Feed Consumption (gram/bird/day)
FSIS	Food Safety Inspection Services
ft	Foot
g	Gram
h	Hours
IACUC	Institutional Animal Care and Use Committee
kcal	Kilocalorie
kg	Kilogram
m	Meters
MBM	Meat and bone meal
mg	Milligram
PC	Positive control
ppm	Parts per million
SBM	Soybean Meal
WB	Woody Breast
WS	White Striping

TABLE OF CONTENTS

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

As the consumer's demand for chicken products continues to rise, so does the drive to create nutritious protein more efficiently. Today's consumers no longer have a high demand for whole carcasses; rather, more emphasis has been placed on value added and convenient products. There is also a renewed interest in the health benefits of chicken compared to other protein sources due to the low dietary fat and high protein content. The overall prices, nutritional facts, and sensory properties have made poultry protein extremely competitive and highly consumed (Larry, 2002; Valceschini, 2006). The consumption of chicken has more than doubled in 2015 compared to 1965 and is projected to triple in 2017 (National Chicken Council, 2017). This shift in the market is driving the industry to produce a rapid growing broiler over a shorter time period at a reduced cost.

Over the last 70 years, the industry has intensely selected numerous meat-type chicken strains for rapid growth, enhanced feed efficiency, and an elevated meat yield. When comparing modern broilers to those of 50 years ago, the growth rate has increased 300%, feed conversion has reduced significantly and body weight has increased ~3.30% over each year (National Chicken Council, 2016). It is suggested that 85 to 90% of these improvements are credited to genetic selection for higher growth rate and feed intake

(Havenstein et al., 2003a,b; Zuidhof et al., 2014). Nutritional and management improvements have also contributed to improvement in growth performance measures.

Today's broilers are marketed at 48 days of age and weigh an average of 2.98 kg/bird, a breast meat yield range of ~24 to 26%, and have a livability rate of ~95.4%, (National Chicken Council, 2016). However, it is believed that this improvement in genetic potential through the selection for elevated levels of breast tissue yield has led to an influx of breast myopathies that are related to a decrease in water holding capacity, increased hardness, and pale color (Dransfield and Sosnicki, 1999; Kuttappan et al., 2012; Petracci et al., 2014).

The improvements in growth rate have driven metabolic demands upward resulting in an increase of metabolic disorders and muscle myopathies (Julian, 2005). Two muscle myopathies that have emerged in recent years are white striping (WS) and woody or "wooden" breast (WB). Recent publications by Kuttappan et al. (2013), Shivo et al. (2014) and Kuttappan et al. (2016) are in agreement that WB and WS are expressed by rapidly-growing and high yielding genetic strains that are raised for a longer period of time. Unfortunately, etiologies of both of these muscle myopathies are still unknown. However, Petracci et al. (2013) reported that broilers marketed with the sole purpose of bearing higher breast yield were observed to have increased incidence of WS. Additionally, Kuttappan et al. (2012b) hypothesized a secondary nutritional deficiency similar to nutritional muscular dystrophy may be present and responsible for WS and WB.

White Striping

White striping has been defined by the presence of white striations that run parallel to the muscle fibers and is commonly found in breast fillets and thighs (Kuttappan et al., 2013).White striping can be first observed on the cranial region of the breast and develops caudally as the severity increases (Kuttappan et al., 2013). Histological lesions observed in WS indicate the occurrence of a degradation process by the loss of cross striations, variability in fiber size, and mild mineralization in muscle fibers (Petracci et al., 2013). Studies have also suggested an increase in eosinophilia, degeneration and lysis of fibers, mononuclear cell infiltration, lipidosis, and interstitial inflammation and fibrosis occur in severe cases of WS (Kuttappan et al., 2013; Petracci et al., 2013, Sihvo et al., 2014). Additionally, compared to a normal breast fillet, WS has resulted in a higher caloric breast fillet which can be attributed to an increase in monounsaturated fat content and a decrease in protein content (Kuttappan et al., 2012b; Kuttappan et al., 2013).

White striping is commonly reported in commercial conditions around the world. Italy has observed WS in 12% of medium sized birds from high yielding broilers strains reared under commercial conditions (Petracci et al., 2013). Similarly, Kuttappan et al. (2012b) reported that under experimental conditions, WS can occur in over 50% of high yielding broiler strains. These studies are in agreement with several authors who found evidence 15 years ago that the selection for higher breast meat yield results in severe changes in protein catabolism, muscle fiber diameter, and sarcolemmal integrity (Dransfield and Sosnicki, 1999; Duclos et al., 2007; Petracci and Cavani, 2012).

A study conducted by Kuttappan et al. (2013) noted that breasts with moderate to severe WS present were correlated with heavier and thicker fillets. Congruently, Brewer et al. (2012) observed a higher correlation between heaver fillet weights and thickness in the cranial region. Observed male broilers exhibited a higher percentage of severe WS when compared to females (Kuttappan et al., 2013). The elevated incident in filets from male broilers can be related to heavier and thicker breast fillets versus those from females.

Although intramuscular deposits similar to WS are favorable in pork and cattle; it has shown to negatively affect consumer acceptability in poultry (Kuttappan et al., 2016). A study conducted by Kuttappan et al. (2012a) reported that 50% of consumers stated they would not purchase a moderate to severe WS effected breast fillet. Consumers disliked the “fatty” or “marbled” appearance, stating that it gave the impression that the breast fillet was “unhealthy” or had less health benefits than a normal fillet. Furthermore, when compared to a normal breast fillet, WS has been observed to possess altered meat quality properties. Petracci et al. (2013) noted a significant impact on marinade uptake, reduction in shear force, and water holding capacity (WHC) properties resulting in higher drip and cook loss. Fillets showing severe WS that result in these altered meat quality properties may be downgraded in commercial plants and not marketed for fresh retailing. If this were to occur, economic damage to the poultry industry would ensue.

Woody Breast

In addition to white striping, woody breast has been characterized by its substantial pale color and rigid breast fillets which are typically firmer upon palpation compared to normal fillets (Sihvo et al., 2014; Mudalal et al., 2014). Often times, a gelatinous exudate of clear or semi clear fluid can also be found on the surface of the breast tissue (Tijare et al., 2016). As WB increases in severity a significant bulge-like ridge begins in the cranial region and extends into the caudal region of the breast fillet allowing for limited or no flexibility. Recent publications have reported that no additional skeletal muscles appear to be affected or any particular ante mortem signs have been associated with this condition. However, histological lesions are present typically in the form of myodegeneration and necrosis, fibrosis, lipidosis, and infiltration of macrophages and lymphocytes (Kuttappan et al., 2013b; Sihvo et al., 2014; Trocino et al., 2015; de Brot et al., 2016; Kuttappan et al., 2016). Similar to WS, increased reports of WB have been expressed in countries around the world. Although the etiology is unknown, there are different hypothesis emerging about the potential causes.

One of the leading hypotheses that pertain to WB and broiler performance is nutritional deficiency. Over the years, nutrition has been widely studied due to its causes on myodegeneration in the muscle structure (Van Vleet and Valentine, 2007). Kuttappan et al. (2012) noted that broilers fed a high-energy and high protein diets experienced an increase in WB incidence. Mutryn et al. (2015) also hypothesized that localized hypoxia, oxidative stress, higher levels of intracellular calcium, and muscle fiber type switching, that is associated with modern fast growing broilers, could be correlated with the WB myopathy.

In the last two decades, continuous genetic selection focused on increasing breast meat yield has resulted in a dramatically larger breast. The increase in tissue mass requires additional nutrients without additional vascularity forcing stress onto the system. The stress may be leading to a reduction of oxygen within the breast tissue, creating a hypoxic state. Bilgili (2013) originally hypothesized that the WB fillet was in a hypoxic state due to a reduction in capillary supply. Mutryn et al. (2015) validated that hypothesis when observing the hypoxia- inducible factor 1 (HIF-1) gene up-regulating several differentiated genes suggesting a hypoxic state within the muscle. During hypoxia, HIF-1 up-regulates the gene, PLOD2, which is responsible for the stiffening of the extracellular matrix as seen in fibrosis (Van der Slot et al., 2003). The up-regulation of PLOD2 leads to an increase in collagen content and the stiff cellular environment (Gilkes et al., 2003). These cellular changes correlate with the histological characteristics that have been associated with WB such as fibrosis (Kuttappan et al., 2016).

Oxidative stress has also been speculated to be a plausible cause of WB due to the increase in the reactive oxygen species (ROS). A major increase in ROS can be detrimental to muscles because of the damaging and altering effects on proteins due to the cytotoxicity (Powers et al., 2010). Reactive oxygen species also have the ability to activate signaling pathways under chronic activation that promote proteolysis and potentially cell death. Additionally, an increase of ROS has the potential to damage the contractile ability of the muscle by impacting calcium myofibril sensitivity (Allen et al., 2008).

With the potential of the contractile ability of the muscle to be injured, the protein makeup must also be considered. A study conducted by Kuttappan et al. (2017) observed 62 proteins that were significantly different between severe WB and a normal breast fillet. A change in the protein expression found in pathways such as mechanistic target of rapamycin (mTOR), gluconeogenesis, and glycolysis were also observed. This change in the protein metabolism could be correlated with the growth rate seen in birds with WB myopathy. Typically muscle post-natal growth occurs through hyperplasia and hypertrophy of satellite cells (Kuttappan et al., 2017). Satellite cells are defined as proliferative, myoblastic cells that lie in the invaginations of the sarcolemma. Often times, satellite cells hyperplasia exceeds hypertrophy. The cells are stimulated to proliferate during muscle damage and repair. Once proliferation and differentiation occurs, cells will fuse into myocytes, myotubules, muscle cells, and ultimately form complete muscle groups. These cells are critical during embryogenesis for muscle development due to the formation of primary and secondary myotubes.

Furthermore, these biological and cellular changes have tremendously impacted overall meat quality. Similar to WS, studies have shown functional properties in WB have been altered as evidenced the increase in fat and decrease in protein content within the breast fillet (Sihvo et al., 2014; Petracci et al., 2014; Soglia et al., 2016 a,b). Mazzoni et al. (2015) also observed an impaired water holding capacity in heavy broilers. Furthermore, Tijare et al. (2016) observed a 4.3% reduced marinade pick up, a 7.0%

increased cook loss, and a 0.1% increase in sarcomere length in WB birds. Sihvo et al. (2014) suggested the reduction in marinade pick up could potentially be attributed to the loss of protein due to the fibrosis found in WB. These abnormalities in meat quality stand to greatly affect the poultry industry, especially the further processing sector and/or food service where breast meat is commonly used (Tijare et al., 2016). Additionally, the breast meat has the potential to be downgraded in the plant, negatively impacting revenue.

The texture of a food product plays a key role in consumer acceptance. A study was conducted using instrumental texture measurements and confirmed that WB affected fillets are harder and chewier compared to normal breast fillets (Chatterjee et al., 2016). Similarly, sensory evaluations revealed WB affected fillets were perceived as more springy and cohesive when compared to normal breast fillets (Sanchez Brambila et al., 2017). However, this was not observed to be uniform throughout the fillet. The ventral portion of the fillet was observed to have a higher level of springiness, hardness and fibrousness when compared to the dorsal sections (Sanchez Brambila et al., 2017). Due to these negative impacts, processing facilities have begun sorting WB fillets from the processing line and using it for ground products (Crews, 2016). In order to understand the impact of WB on ground products, a study was conducted on chicken nuggets using ground WB compared to normal ground breast tissue. Qin (2013) reported no significant differences in shear force, binding strength, or cook loss between WB and normal breast fillets. These results suggest that ground WB shows different properties when compared to intact WB fillets. Sanchez Brambila et al. (2017) also reported that grinding reduces the overall intensity of sensory texture regardless of attribute when compared to a cooked intact WB fillet.

The negative impact WS and WB has upon meat quality properties, consumer acceptability, and further processing could potentially cost the industry tremendous losses in revenue. The Wall Street Journal reported that 5 to 10% of boneless, skinless breast meat produced could potentially have WB (Gee, 2016) which is currently being sold at a discounted price or used for further processed products. The United States produces 12 billion pounds of breast meat per year and stands to lose over $200 million per year due to the incidence of WS and WB (Kuttappan et al., 2016). This immense loss in revenue can be attributed to a decrease in yield, increase in downgrades and discarded meat (Kuttappan et al., 2016). Unfortunately, the loss in revenue has the potential to increase with the recent ruling by FSIS (USDA, 2017). The ruling states that inflamed WB, which includes; swollen breast tissues, scattered blood spots, or presence of gelatinous fluid, will be considered adulterated. Any adulterated meat is considered unfit for human food and will either need to be trimmed or completely discarded (USDA, 2017). Extensive research is being done in all areas to mitigate this issue and increase the percentage of saleable meat.

Amino Acid Density

As the broiler has evolved over the past 50-60 years, there have been notable increases in live performance weights and meat yields. These differences can be attributed to many factors including nutrition and genetic selection. In order to maintain this rapid growth rate, it is essential that the supply of amino acids and energy is maintained throughout all phases of broiler growth. Amino acids are considered to be the building blocks of muscle development and are sourced from the animal's diet. Over 200 AA are found in nature but only 22 are considered to play a role as building blocks of protein and polypeptides. These 22 amino acids can be further subdivided into two categories: essential (EAA) and non-essential (NEAA). Essential amino acids must be provided to the animal through the diet to meet the optimal needs when rates of utilization are greater than rate of synthesis (Wu, 2009). Non-essential amino acids can be synthesized de novo to meet the body's optimal requirement. In poultry, the first limiting amino acid is methionine, followed by lysine, and threonine in a corn/soybean meal based diet (Vieira et al., 2016). Amino acids have the ability to regulate key metabolic pathways, optimize muscle growth and enhance protein synthesis (Wu, 2009). Due to its large mass, skeletal muscle is the largest reservoir of both peptide- bound and free amino acids within the broilers body (Davis and Fiorotto, 2009).

Amino acids have also shown to impact growth rate by influencing satellite cell development. The role of satellite cells during skeletal growth is to proliferate and donate nuclei to the growing myofiber (Pophal et al., 2004). The first phase of increased myofiber growth occurs early in life and is commonly characterized by the high level of satellite cells. Thus, the impact of nutrition on satellite cells mitotic activity in the early stages is critical (Pophal et al., 2004). A study conducted by Powell et al. (2013) reduced the concentration of methionine and cysteine at varying levels in order to decrease the total sulfur amino acid concentration that was available to the cells. The treatment containing 0/0 mg/L Met/Cys reduced satellite cell activity in the pectoralis major by suppressing cell proliferation and differentiation. Powell et al. (2013) suggested that satellite cells are likely to respond to a critical range of nutritional availability. The alteration of satellite cell activity by the use of amino acids can impact muscle fiber formation and ultimately muscle mass accretion in the pectoralis major.

It has been well established that adjustment of dietary amino acid density influences observed broiler performance. Corzo et al. (2010) reported that when Cobb 500 males were fed a diet with a target increase of 0.1% dLys later on in the feeding phases; live performance, breast meat yield and abdominal fat content benefitted greatly. Congruently, a study conducted by Kidd et al. (2005) observed broilers fed a lower amino acid density diet with a target of 0.1% in reduction for dLys, experienced a reduction in body weight, feed conversion and carcass yield. However, Summers et al. (1989) noted that when broilers are feed restricted at 21 days, no negative impact is observed on body weight at day 42 when compared to the control birds. Similarly, Leeson et al. (1990) reported that broilers can withstand a period of undernutrition without a loss in market weight or feed efficiency. These results can be attributed to compensatory gain which has been studied in a number of animals as a means of enhancing weight gain. However, in most cases weight gain equaled to that of the control animals but feed utilization was enhanced (Summers et al., 1990). Although, when developing a feeding program implementing this technique; the severity, duration of nutrient restriction, and the broilers stage of maturity should be considered (Wilson and Osbourn, 1960).

Lysine

The ratio of amino acids within the diet is also essential in broiler growth and muscle development. Every amino acid has a different role and efficiency within the body. Lysine typically represents the basis to which all other amino acids are related to achieve an ideal balance otherwise known as the ideal protein concept (Corzo et al., 2002). Lysine is particularly important due to its association with protein accretion and growth (Waldroup et al., 1976; Han and Baker, 1994). It is also known to exhibit specific effects on carcass composition such as increasing carcass protein and decreasing carcass fat (Bedford and Summers, 1985). Furthermore, when fed at levels above the requirement, lysine can significantly improve breast meat yield (Hickling et al., 1990; Moran and Bilgili, 1990). Leclercq (1998) reported that a deficiency in lysine reduces the *pectoralis major* growth without significantly impacting other muscle groups. Therefore, this reduction in growth can lead to a conclusion that the pectoralis major is more sensitive to lysine intake than other muscles (Leclercq, 1998).

Arginine

While lysine falls into the category of an essential amino acid, a new sector of amino acids has emerged over the years known as functional amino acids. Functional amino acids can be classified as amino acids that regulate key metabolic pathways to improve health, growth, and development (Wu, 2010). These include arginine, cysteine, glutamine, leucine, proline, and tryptophan. Supplementing these amino acids in a broiler diet can be beneficial for optimizing efficiency of metabolic transformations to enhance muscle growth (Wu et al., 2004; Wu, 2009; Wu et al., 2010). However, arginine has an important interrelationship with Lysine within a broiler diet due to its similar chemical and structural properties. Arginine and lysine have an antagonistic relationship which is defined as interference in the metabolism of one amino acid caused by the intake of the other amino acid (Harper et al., 1970). Arginine and lysine compete for renal tubular reabsorption (Boorman et al., 1968) and an excess of lysine will enhance degradation and increase urinary loss of arginine in renal arginase activity (Austic and Calvert, 1981). In order to combat this antagonism, supplementation of the amino acid that is being antagonized is required (Harper et al., 1970).

The arginine requirement in a diet can be influenced by many factors including rate of *de novo* synthesis and oxidation as well as the synthesis of additional metabolites (Ball et al., 2007). Arginine is responsible for a multitude of biological and physiological processes including the activation of mTOR signaling, antioxidant properties, and immune function. It is also produces nitric oxide (NO), an important signaling molecule and regulator of nutrient metabolism (Wu, 2009). Chickens are unable to synthesize arginine *de novo* resulting in an absolute requirement within the diet (Tamir and Ratner, 1963). However, Dietert et al. (1994) noted that the levels of arginine recommended for maximal growth may not be adequate enough to reach maximal NO production. The concentration of arginine that maximizes NO production in broilers macrophages is approximately 0.40mM. This is well above the 0.25mM arginine concentration in broilers receiving arginine supplementation for maximal growth (Dietert et al., 1994).

Additionally, arginine is the substrate for biosynthesis of several molecules including protein, creatine, glutamate, ornithine (Fouad et al., 2012) and endothelial NO synthase (eNOS). Endothelial NO synthase, a potent vasodilator , at certain levels can signal the pulmonary artery smooth muscle cells to relax, therefore maintaining vascular tone (Tan et al., 2005; Dudzinski and Michel, 2007; Bautista-Ortega and Feria, 2010). Bautista-Ortega and Feria (2010) noted that supplementing arginine and antioxidants improved the pulmonary vascular performance of hypoxic broilers. Similarly, Wideman et al. (1995) observed supplemental dietary arginine improved flow-dependent vasodilation and reduced the pulmonary vascular resistance. This improvement reduces vasodilation and in turn, increases blood pressure and blood flow.

Arginine has shown to influence and improve carcass quality and yield which is crucial for producers and is intensely researched. Arginine levels fed above the NRC levels during the starter phase may be beneficial in improving muscle development and achieving maximum breast yield (Fernandez et al., 2009). Fernandez et al. (2009) observed a significant increase in breast weight and myofiber diameter of broilers fed diets supplemented with arginine at 1.30 dArg:dLys exceeding the recommended ratio of 1.05 and 1.02. The increase in myofiber diameter can be indicative of meat quality due to the association with meat firmness and resistance. Broilers that experience high muscle yield have higher susceptibility to muscle damage (Velleman, 2007). Thus, the improvement in muscle protein deposition is monitored to evaluate meat quality. Furthermore, it can be hypothesized the supplementation of arginine contributed to the muscle hypertrophy during the starter phase by improving nutrient usage. Similarly, when included at a level of 2.5 times the NRC level, a higher concentration of serum total protein was observed in heavier broilers (Emadi et al., 2011). This may be due to an increased demand for lean tissue maintenance and protein turnover (Corzo et al., 2005). Congruently, Khajali et al. (2011) noted a reduction in carcass and breast yield in broilers fed an arginine deficient diet. Therefore, supplementation of arginine is beneficial in enhancing performance, carcass quality, and carcass yield (Fouad et al., 2012).

The improvements in growth rate and the rise in nutritional deficiencies of amino acids have unfortunately led to an increase in disease susceptibility and a reduction in lymphocyte populations (Dietert and Austic, 1994; Swaggerty et al., 2009). To combat this negative impact, improving the immune system through nutrients is considered a practical and efficient practice (Kidd, 2004). Arginine has shown to be beneficial in improving immune response. Kwak et al. (1999) noted that feeding an arginine deficient diet resulted in a significant decrease in relative weight of the spleen, thymus, and bursa. Congruently, Ruiz-Feria (2009) observed enhanced antibody titers to sheep red blood cells when arginine was supplemented in a broiler diet. Furthermore, the inclusion of arginine above the NRC required levels significantly enhances B cells and T lymphocytes (Abdukalykove et al., 2008).

Antioxidants

Antioxidants are essential in the maintenance of high growth levels and immune-competence in poultry production (Surai, 2007). These can include vitamin E, carotenoids, flavonoids, and ascorbic acid or vitamin C. It has been suggested that the balance of antioxidant-prooxidant in each cell is responsible for many major physiological functions (Surai, 2007). This balance can be affected by dietary supplementation or adversely poor nutrient intake. If an imbalance does occur, the potential for oxidative stress and damage to biological molecules and membranes arises (Panda and Cherian, 2014). Therefore, maintaining dietary intake of antioxidants is important to prevent oxidative damage.

Antioxidants possess many important attributes; however, one of the most important roles is the maintenance and neutralization of harmful molecules. These molecules include free radicals that can be defined as atoms containing one or more unpaired electrons (Surai, 2002). These are derived from reactive oxygen species (ROS) and reactive nitrogen species (RNS). Although these two elements are essential, in certain conditions, they can be converted into unstable free radicals causing potential damage to DNA, proteins, lipids, and carbohydrates (Surai, 2002). However, due to the physiological mediation and signaling capabilities of ROS, the complete removal could be detrimental to the body (Surai, 2002). Reactive oxygen species are produced by the immune system as part of its defense function and are crucial in the destruction of pathogens. Macrophages bind, internalize, and degrade foreign antigens with the aid of ROS. With the complete elimination of ROS, it exposes the cell to potential pathogens or death. However, chronic production of ROS can cause oxidative damage (Surai, 2002). The chronic production can lead to enhanced superoxide production in heat stress broilers ultimately resulting in a significant reduction in body weight gain and reduced carcass yields (Ali et al., 2008).

Antioxidants possess the ability to react with free radicals resulting in a less reactive molecule and a delayed oxidation of biological molecules. All antioxidants work together to prevent the damaging effects and toxic metabolism of free radicals. This is commonly referred to as the "antioxidant system" (Surai, 2007). The antioxidant system aids in controlling the formation of free radicals, where deficiencies in one antioxidant impacts the efficiency of others. The antioxidant system is based on three major levels of defense. The first level is the preventative level composed of metal binding proteins that prevent free radical formation (Fellenberg and Speisky, 2007). The second level of defense is preventing the damage from spreading by chain breaking antioxidants such as Vitamin A, E, and C. The last level of defense handles damaged molecules as a result of free radicals and toxic products by repairing and removing the impaired molecules through enzymatic systems (Surai, 2007). Damage to organs and cells can occur if free radical formation dramatically increases and forces the antioxidant system into a high stress condition. To combat this detrimental damage, dietary supplementation of natural antioxidants and minerals such as vitamin C may be beneficial (Surai, 2007). However, this poses a challenge for nutritionists due to the high cost of antioxidant inclusion in the diet.

Selenium (Se) is commonly added in a broiler diet in the trace mineral premix and may aid in supporting other antioxidants such as vitamin C in the defense against oxidative stress. Selenium has a special role within the natural antioxidants by aiding in the regulation of various physiological processes (Surai, 2002). Selenium can exist as organic and inorganic forms. Historically the most common Se supplements currently used in a broiler diet are selenite and selenate, both inorganic forms of Se (Surai, 2002). However, the formation of an organic source of Se in the form of SeMet is gaining in popularity due to its antioxidant properties (Schrauzer, 2000). Unfortunately, Se has its limitations due to toxicity, interactions with other minerals, and the inability to build and maintain Se reserves within the body. While selenium is involved in a multitude of processes, it is important to observe the interrelationship between selenium and vitamin E. Vitamin E is unable to destroy all metabolic peroxides so a second line of defense from selenium is needed. McDowell (1992) noted that Vitamin E and selenium can act as a sparing mechanism for one another. Through this process, selenium can act alongside vitamin E and C to reduce cellular oxidative stress (Puvaca and Stanacev, 2011).

Although selenium possesses antioxidant properties, the most important and well characterized natural antioxidants include vitamin E and vitamin C. Vitamin E has been widely used in poultry diets and has increased tremendously over the recent years (Surai, 1999). This increase is thought to protect birds that may come into contact with stressors within the commercial production system. Although vitamin E has tremendous antioxidant properties, it plays a supporting role to vitamin C in the reduction of oxidative stress. Vitamin E originates from plants and is considered a fat-soluble vitamin and can commonly be found in feedstuffs such as wheat, barley, and corn (Surai, 1999; Kuttappan et al., 2012b). Vitamin E has been observed to prevent oxidative damage induced by free radicals by trapping reactive oxyradicals (Surai, 1999). This action thus prevents damage to DNA, proteins, and polyunsaturated fatty acids present on the cell membrane (Mezes et al., 1997). In some cases, Vitamin E has also been used as a vaccine adjuvant to aide in improving vaccination efficiency (Surai, 2007). In regards to carcass quality, dietary supplementation of vitamin E retards oxidation in the meat and enhances the shelf life. Congruently, supplementation of vitamin E may be necessary to prevent flavor degradation by lipid oxidation (Wood and Enser, 1997). Furthermore, Hidiroglou et al. (1992) suggested that certain factors including the presence of other dietary antioxidants such as vitamin C and the degree of oxidation of fat present in the diet could affect the variability of Vitamin E in feedstuffs.

Vitamin C

Coupled with Vitamin E as one of the most important natural oxidants, is Vitamin C or ascorbic acid. Vitamin C serves as an elector donor in a multitude of enzymatic reactions and is essential in the biosynthesis of collagen, making it one of the most crucial antioxidants (Ishikawa et al., 2013). Vitamin C has the ability to act as an antioxidant in two ways. First, it can serve as a dietary antioxidant due to its capability to protect cellular components from free radical damage (Florou-Paneri et al., 2005). Second, it can readily oxidize to dehydroascorbic acid, this action being reversible and forming a redox system (Whitehead and Keller, 2003). Furthermore, Vitamin C also possesses the ability to restore the antioxidant capability of oxidized Vitamin E (Guney et al., 2007). Due to these properties, vitamin C has a tremendous impact upon oxidative stress.

Under normal conditions, vitamin C can be sufficiently synthesized by the broiler to meet its physiological needs (Marks, 1975). However, in a high stress environment exogenous supplementation may be beneficial to maintain growth and development. High stress environments may include heat stress, increase in humidity, and parasitic infection (McDowell, 1989). Broilers under stress will produce corticosterone, the main hormone associated with stress in chickens. A rise in corticosterone levels can cause secondary effects such as immunosuppression and the production of adrenaline. Vitamin C has been observed to be closely associated with corticosterone production (Whitehead and Keller, 2003). A rise in production will deplete ascorbic acid; however a study conducted by Pardue et al. (1985) noted that dietary supplementation of Vitamin C limited corticosterone concentrations in stressed broilers. Furthermore, Sahin et al. (2003) noted that the inclusion of vitamin C on heat stressed broilers improved live weight gain, feed efficiency, and carcass yields. These data suggest that vitamin C may offer potential protection against heat stress related depression in performance (Sahin et al., 2003).

This protection may be beneficial when external stressors, specifically heat stress, leads to a rise in free radicals and other reactive oxygen species (Elstner, 1991; Halliewell et al., 1992). A rise in ROS could result in an imbalance between the oxidation and antioxidant defense system. The rise in ROS leads to lipid peroxidation and oxidative damage to proteins and DNA, commonly known as oxidative stress (Gutteridge and Halliwell, 1989; Droge, 2002). Oxidative stress is considered an important factor of biological damage and the cause of many conditions impacting poultry growth (Avanzo et al., 2001; Iqbal et al., 2001). Oxidative stress results in damage to body proteins, lipids, and DNA (McCall and Frei, 1999) and causes a reduction in growth performance and health of the bird (Lykkesfeldt and Svendsen, 2007). Sandercock et al. (2001) speculated the change in redox balance was related to the impaired muscle membrane integrity of the breast muscle in heat stressed broilers. Oxidative damage can potentially be minimized by the antioxidant defense mechanisms as described earlier. Therefore, optimizing and maintaining a balance in antioxidants in the diet is essential to minimizing damage within the body (Panda and Cherian, 2014).

Additionally, stressors can also potentially trigger conditions such as pulmonary hypertension syndrome or ascites in broilers. The etiology to this problem is complex, however, hypoxia is observed to be major predisposing factor (Ruiz-Feria, 2009). Bottje and Wideman (1995) noted that increases in free radical production occur during systemic hypoxia. Congruently, a study conducted by Bautista-Ortega et al. (2010) reported that diets supplemented with arginine, vitamin E, and vitamin C significantly improved the pulmonary vascular performance of hypoxic broilers. This could potentially be attributed to the synergistic role the antioxidant vitamins and arginine had upon the NO bioavailability (Bautista-Ortega et al., 2010).

As mentioned earlier, corticosterone is released in response to stress in broilers and can have a significant immunosuppressive effect. Vitamin C has historically been thought to be essential in the function of the immune system by enhancing neutrophil production and protection against free radicals (Bendich et al., 1986). It has been found to be present in high concentrations in leukocytes and is highly utilized during and infection (Thomas and Holt, 1978). Previous research has shown that broilers challenged with inactive Newcastle disease virus were observed to improve antibody formation when fed a diet with vitamin C in the early stages of growth (Franchini et al., 1994). Conversely, in the presence of heat stress, dietary supplementation of vitamin C has been observed to enhance the haemagglutination titer in broilers vaccinated with Newcastle disease (Bashir et al., 1998). Therefore, the supplementation of vitamin C into the diet may be beneficial in immune response against diseases (Wu and Lin, 2000).

Advancements in genetic selection, nutrition and management practices have produced a rapid growing broiler with improvements in growth performance and carcass yield. These improvements have allowed the industry to meet the rising demands of the consumer. Unfortunately, unforeseen side effects such as the presence of white striping and wood breast have occurred. These muscle myopathies pose a tremendous problem for the industry by immense revenue losses. Nutrition may be one solution to aid in the reduction of these muscle myopathies. Potential strategies can include a reduction in amino acid density, supplementation of arginine, and the inclusion of vitamin C. The reduction in amino acid density early on in growth may allow for satellite cells to recover and repair from the rapid growth experienced during the first 2 weeks of life. Additionally, the inclusion of arginine could aid in the reduction of the hypoxic state of the woody breast muscle by decreasing vasodilation and improving blood flow throughout the muscle. Furthermore, the inclusion of vitamin C as a powerful antioxidant has the potential to reduce oxidative stress within the muscle and protect the muscle cells from free radical damage. Therefore, the aim of this present research program was to evaluate, through a series of experiments, the impact of dietary supplementation on the incidence and severity of woody breast and white striping.

CHAPTER II

EVALUATION OF DIFFERENT DIETARY ALTERATIONS IN THEIR ABILITY TO MITIGATE THE INCIDENCE AND SEVERITY OF WOODY BREAST AND WHITE STRIPING IN COMMERCIAL MALE BROILERS

Introduction

As the public's demand for highly nutritious animal protein sources increases, so does the drive for increasing the amount of meat produced in a rapid and efficient manner. From day-of-hatch to market-age, the broiler is fed a series of diets that are balanced in regards to the following nutrient classes: energy (carbohydrates and lipids), digestible amino acids (dAA), minerals and vitamins. Protein accretion, in the form of muscle tissue, is incredibly dependent on the dietary balance of compounds (i.e. nutrients) within each of these classes. The growth and production responses to the nutritional compounds in each of the previously mentioned classes are what define nutritional requirements.

Over the past 70 years, the broiler industry has intensely selected a number of meat-type chicken strains, with the result that today's broiler genetics have tremendous growth, feed efficiency and meat yield potential. In a retrospective evaluation of broiler genetics from 1957 to 2005 Havenstein et al. (2003a,b) and Zuidhof et al. (2014) determined that both genetic selection and nutrition have had a significant effect upon the production efficiency and meat yield of the modern broiler, where the broiler's growth rate has increased over 300%, body weight has increased ~3.30% year-over-year and feed conversion has dropped ~2.55% year-over-year. In fact, these authors suggest that approximately 85 to 90% of improvements are reportedly due to genetic selection for higher growth rate and feed intake. Based on an average straight-run growout for small to large bird programs, today's broilers are marketed at ~48.8 days-of-age, weigh ~2.98 kg/bird, have an average daily gain (ADG) of ~61.1 g/d, a breast meat yield range of ~24 to 26%, have a livability of ~95.4%, and are raised at a stocking density of ~827 cm2/bird (National Chicken Council, 2016). Even though these statistics are derived from the top 25% of broiler complexes within the USA, the genetic potential of the commercial broiler is much greater. Modeling this potential in a four-phase growout program, feeding high-yielding, male broilers a dAA density that was 110% over that of commercial dLys levels from 0 to 42 days-of-age resulted in a BW of 3.77 kg/bird, an ADG of 89.8 g/day, a 1.60 FCR, and a >95% incidence of severe muscle myopathies at processing (unpublished data from this lab). It is extremely important to note that these data were generated under special circumstances, and in no way, reflect current nutritional practices in the US commercial broiler industry. Regardless, these and other data in the literature indicate that while the broiler has the genetic potential for optimal performance, the propensity for a higher incidence of muscle myopathies can go up when a higher plane of nutrition is fed (i.e. dAA).

Together, genetic selection (i.e. for growth, feed conversion and higher white meat yield), management and nutrition have all had a positive influence on the final processing weight of broilers. Consequently, these have also increased the degree of myodegeneration, fibrosis, lipidosis, and regenerative changes within *Pectoralis major* filets (Bowker and Zhuang, 2017; Kuttappan et al., 2016; Petracci and Cavani, 2012). These changes have resulted in producers, customers and consumers observing an alteration in the quality of breast muscle tissue from the perspective of appearance, touch and chewy texture (Gee, 2016). Today, the two muscle quality issues that have garnered a majority of attention are white striping (WS) and woody breast (WB).

White striping in broilers is characterized by white striations that run parallel to the muscle fibers; these can be visualized on the surface of breast, thigh, and tenderloin (Kuttappan et al., 2013; Petracci et al., 2014). This myopathy is not something that has recently appeared in broilers. Between 2012 and 2015, the incidence of severe WS dramatically increased from 5 to 29% of the broiler flocks/ breast meat (Kuttappan et al., 2012b; Petracci et al., 2013; Owens and Alvarado, 2015; Russo et al., 2015; Bowker and Zhuang, 2016; Tijare et al., 2016). Looking at the components of the *Pectoralis major* filet, Kuttappan et al. (2012b); Kuttappan et al. (2013) and Petracci et al. (2014) reported that white striping results in higher fat content and a lower, altered protein content and profile. In addition to a macro and microscopic alteration in the muscle's appearance (Kuttappan et al., 2009; Ferreira et al., 2014; Petracci et al., 2014; Bowker and Zhuang, 2016), changes in protein content and functionality translate into significantly higher cook losses and reduced marinade uptake (Petracci et al., 2013; Petracci et al., 2014; Tijare et al., 2016). This is a major concern for divisions within a processing plant that "further processes" meat into other high-value products (Petracci et al., 2013; Mudalal et al., 2014; Tijare et al., 2016). Aside from the scientific aspect of how changes in protein functionality affects further processing, surveys indicate that approximately half of all consumers (i.e. the general public that shops at grocery stores) would be less likely to purchase breast filets that contain WS (i.e. are striated or have a “fattier” appearance; Kuttappan (2012a)).

Like WS, WB is reportedly linked to fast growth rate and an increase in breast meat yield (Sihvo et al., 2014); it is found in varying degrees of severity and is characterized by changes in texture, fibrosis of the breast muscle, pale color, and a gelatinous exudate on the surface of the breast tissue (Tijare et al., 2016). Bowker and Zhuang (2017) recently reported on the compounded incidence of WB incidence across different WS scores; essentially, the higher the severity of WS, the higher the incidence of WB. The affected fillets have a reduced marinade uptake and higher cook loss due to fibrosis and an alteration in the sarcoplasmic, myofibrillar and stromal proteins that are expressed in the breast meat (Sihvo et al., 2014; Barbut, 2015; Mudalal et al., 2014). Like WS, the presence of WB in a breast filet can be perceived differently by the consumer as chewy/ rubbery, tough, crunchy, and/or gristly (Gee, 2016; Solo, 2016). There are several general theories to the cause of WS and WB (i.e. rapid growth, genetic selection, poor vasculature relative to tissue type, and possible secondary nutrient deficiencies that are brought about by rapid growth), but none has been definitively identified as the causative agent. Utilizing some basic knowledge of muscle biology, physiology, nutrition, and general production, the goal of this study was to evaluate nutritional strategies that may reduce or eliminate WS and WB, all while maintaining growth performance.

Materials and Methods

Experimental Setup and Dietary Treatments

To evaluate the multiple dietary strategies that might mitigate the incidence and severity of WB and WS, six treatments were conceived and evaluated using a randomized block design. Relative to a standard set of commercial broiler diets that were fed until 45 days-of-age in a four-phase growout (Treatment 1; starter, grower, finisher and withdraw), the strategies investigated were: 1. Increasing the level of digestible Arginine: digestible Lysine (dArg: dLys) in the diet from ~111% to between 120 and 125% (Treatment 2), 2. Supplementing vitamin C into the diets at 94.4 mg vitamin C/ kg feed (100 ppm on a product basis; Treatment 3), 3. Increasing the vitamin premix supplementation two-fold (Treatment 4), 4. Reducing the digestible amino acid density in only the grower phase by 15%, and feeding the exact same starter, finisher and withdraw diets that were fed to treatment 1 (Treatment 5), and 5. Combining the four strategies just mentioned (Treatment 6). A diagram of the experimental setup is in Table 1.

Prior to formulating and mixing the feeds (Tables 1-4), the ingredients (corn, SBM, DDGS, MBM) were all analyzed for apparent metabolizable energy (AME), total and digestible amino acids, proximates (crude protein, fat, fiber, moisture and ash), and both total and phytate phosphorous using Adisseo's NIR platform, PNE (Precise Nutrition Evaluation). After mixing, the treatment feeds were conditioned and pelleted at 70°C and fed *ad libitum* as crumbles in the starter and pellets in the grower, finisher and withdraw phases.

Table 1. Starter dietary formulations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (1 to 12 days-of-age)

Ingredient	Control[1]	High Arginine	Vitamin C	2X Vitamin[2]	Reduced AA[3]	Combin-ation[2]
	------------------------------ % ------------------------------					
Corn	53.15	53.15	53.15	53.15	53.15	53.15
Soybean Meal	35.55	35.55	35.55	35.55	35.55	35.55
Corn DDGS	5.00	5.00	5.00	5.00	5.00	5.00
Soybean Oil	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	1.21	1.21	1.21	1.21	1.21	1.21
Salt, NaCl	0.51	0.51	0.51	0.51	0.51	0.51
Monocalcium Phosphate	0.83	0.83	0.83	0.83	0.83	0.83
DL-Methionine, 99%[4]	0.34	0.34	0.34	0.34	0.34	0.34
L-Lysine HCl, 78.8%[5]	0.28	0.28	0.28	0.28	0.28	0.28
L-Threonine, 98.0%[5]	0.11	0.11	0.11	0.11	0.11	0.11
L-Valine, 96.5%[5]	0.07	0.07	0.07	0.07	0.07	0.07
Choline-Cl, 60%	0.08	0.08	0.08	0.08	0.08	0.08
Selenium Premix, 500 ppm[6]	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[7]	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[8]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin E, 500 IU/g	0.02	0.02	0.02	0.02	0.02	0.02
Phytase[9]	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[5]	--	0.10	--	--	--	0.10
Vitamin C[10]	--	--	0.03	--	--	0.03
Vitamin Premix	0.02	0.02	0.02	0.04	0.02	0.04
Cellulose, Filler (wt: wt)[11]	0.15	0.05	0.12	0.13	0.15	--
Nutrient	Calculated Nutrient Content, %					
AME, kcal/kg	3025	3025	3025	3025	3025	3025
Protein[12]	23.08	23.29	23.08	23.08	23.08	23.28
dLys	1.20	1.20	1.20	1.20	1.20	1.20
dMet	0.64	0.64	0.64	0.64	0.64	0.64
dSAA	0.90	0.90	0.90	0.90	0.90	0.90
dThr	0.78	0.78	0.78	0.78	0.78	0.78
dArg	1.35	1.45	1.35	1.35	1.35	1.45
Vitamin C, mg/kg	--	--	94.38	--	--	94.38
Calcium	0.95	0.95	0.95	0.95	0.95	0.95
Non-Phytate Phosphorus	0.48	0.48	0.48	0.48	0.48	0.48
Total Phosphorus	0.62	0.62	0.62	0.62	0.62	0.62
Sodium	0.22	0.22	0.22	0.22	0.22	0.22
Nutrient	Analyzed Nutrient Content, %					
Fat	5.8	5.7	5.8	5.8	5.9	6.1
Crude Fiber	2.5	3.0	12.3	4.0	3.3	3.0
Ash	4.9	4.9	5.0	4.7	4.8	5.3
Dry Matter	88.4	88.7	89.1	87.9	88.5	88.2

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

Table 1. Continued
[2] Vitamin premix added at this rate yields 15 400 IU vitamin A, 11 000 ICU vitamin D3, 110 IU vitamin E, 3 mg vitamin K-3, 0.02 mg B12, 13.2 mg riboflavin, 77 mg niacin, 19.8 mg d-pantothenic acid, 1.76 mg folic acid, 5.5 mg pyroxidine, 3.08 mg thiamine, 0.16 mg biotin per kg diet
[3] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 12-24). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.
[4] Rodimet®NP99, Adisseo France SAS.
[5] Ajinomoto Heartland Inc., Eddyville, IA
[6] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.
[7] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.
[8] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.
[9] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.
[10] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.
[11] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine, Vitamin C and/ or vitamin premix.
[12] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 2 and 6 were adjusted based on the amount of supplemental L-Arg and its level of CP

Table 2. Grower dietary formulations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (13 to 24 days-of-age)

Ingredient	Control[1]	High Arginine	Vitamin C	2X Vitamin[2]	Reduced AA[3]	Combin-ation[2]
	------------------- % -------------------					
Corn	58.16	58.16	58.16	58.16	66.62	66.62
Soybean Meal	29.13	29.13	29.13	29.13	21.95	21.95
Corn DDGS	6.00	6.00	6.00	6.00	6.00	6.00
Soybean Oil	2.00	2.00	2.00	2.00	2.00	2.00
Meat & Bone Meal	0.83	0.83	0.83	0.83	0.89	0.89
Limestone	2.07	2.07	2.07	2.07	0.75	0.75
Salt, NaCl	0.44	0.44	0.44	0.44	0.44	0.44
Monocalcium Phosphate	0.24	0.24	0.24	0.24	0.29	0.29
DL-Methionine, 99%[4]	0.30	0.30	0.30	0.30	0.22	0.22
L-Lysine HCl, 78.8%[5]	0.26	0.26	0.26	0.26	0.25	0.25
L-Threonine, 98.0%[5]	0.10	0.10	0.10	0.10	0.08	0.08
L-Valine, 96.5%[5]	0.04	0.04	0.04	0.04	0.01	0.01
Choline-Cl, 60%	0.07	0.07	0.07	0.07	0.11	0.11
Selenium Premix, 500 ppm[6]	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[7]	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[8]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin E, 500 IU/g	0.02	0.02	0.02	0.02	0.02	0.02
Phytase[9]	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[5]	--	0.13	--	--	--	0.11
Vitamin C[10]	--	--	0.03	--	--	0.03
Vitamin Premix	0.02	0.02	0.02	0.04	0.02	0.04
Cellulose, Filler (wt: wt)[11]	0.13	--	0.10	0.11	0.16	--
Nutrient	Calculated Nutrient Content, %					
AME, kcal/kg	3085	3085	3085	3085	3085	3085
Protein[12]	21.60	21.84	21.60	21.60	18.91	18.91
dLys	1.09	1.09	1.09	1.09	0.93	0.93
dMet	0.58	0.58	0.58	0.58	0.48	0.48
dSAA	0.97	0.97	0.97	0.97	0.82	0.82
dThr dArg	0.72 1.25	0.72 1.37	0.72 1.25	0.72 1.25	0.61 1.06	0.61 1.17
Vitamin C, mg/kg	--	--	94.38	--	--	94.38
Calcium	0.85	0.85	0.85	0.85	0.85	0.85
Non-Phytate Phosphorus	0.43	0.43	0.43	0.43	0.43	0.43
Total Phosphorus	0.55	0.55	0.55	0.55	0.54	0.54
Sodium	0.21	0.21	0.21	0.21	0.21	0.21
	Analyzed Nutrient Content, %					
Fat	5.6	5.7	5.7	5.9	4.7	4.5
Crude fiber	3.0	2.5	2.2	2.8	2.3	2.8
Ash	5.0	5.0	5.1	4.9	4.7	4.6
Dry matter	88.9	89.1	88.3	88.8	88.7	88.8

Table 2. Continued

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[2] Vitamin premix added at this rate yields 15 400 IU vitamin A, 11 000 ICU vitamin D3, 110 IU vitamin E, 3 mg vitamin K-3, 0.02 mg B12, 13.2 mg riboflavin, 77 mg niacin, 19.8 mg d-pantothenic acid, 1.76 mg folic acid, 5.5 mg pyroxidine, 3.08 mg thiamine, 0.16 mg biotin per kg diet

[3] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 12-24). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[4] Rodimet®NP99, Adisseo France SAS.

[5] Ajinomoto Heartland Inc., Eddyville, IA

[6] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[7] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[8] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.

[9] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[10] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[11] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine, Vitamin C and/ or vitamin premix.

[12] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 2 and 6 were adjusted based on the amount of supplemental L-Arg and its level of C

Table 3. Finisher dietary formulations, calculated nutrient content, and analyzed nutrient content treatment diets fed to male broilers (25 to 36 days-of-age)

Ingredient	Control[1]	High Arginine	Vitamin C	2X Vitamin[2]	Reduced AA[3]	Combin-ation[2]
	-- % --					
Corn	62.23	62.23	62.23	62.23	62.23	62.23
Soybean Meal	24.29	24.29	24.29	24.29	24.29	24.29
Corn DDGS	7.00	7.00	7.00	7.00	7.00	7.00
Soybean Oil	2.26	2.26	2.26	2.26	2.26	2.26
Meat & Bone Meal	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.71	0.71	0.71	0.71	0.71	0.71
Salt, NaCl	0.41	0.41	0.41	0.41	0.41	0.41
Monocalcium Phosphate	0.02	0.02	0.02	0.02	0.02	0.02
DL-Methionine, 99%[4]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl, 78.8%[5]	0.25	0.25	0.25	0.25	0.25	0.25
L-Threonine, 98.0%[5]	0.09	0.09	0.09	0.09	0.09	0.09
L-Valine, 96.5%[5]	0.02	0.02	0.02	0.02	0.02	0.02
Choline-Cl, 60%	0.07	0.07	0.07	0.07	0.07	0.07
Selenium Premix, 500 ppm[6]	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[7]	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[8]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin E, 500 IU/g	0.02	0.02	0.02	0.02	0.02	0.02
Phytase[9]	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[5]	--	0.11	--	--	--	0.11
Vitamin C[10]	--	--	0.03	--	--	0.03
Vitamin Premix	0.02	0.02	0.02	0.04	0.02	0.04
Cellulose, Filler (wt: wt)[11]	0.16	0.05	0.13	0.14	0.16	--
Nutrient	Calculated Nutrient Content, %					
AME, kcal/kg	3155	3155	3155	3155	3155	3155
Protein[12]	19.79	20.00	19.79	19.79	19.79	20.00
dLys	0.98	0.98	0.98	0.98	0.98	0.98
dMet	0.52	0.52	0.52	0.52	0.52	0.52
dSAA	0.76	0.76	0.76	0.76	0.76	0.76
dThr	0.66	0.66	0.66	0.66	0.66	0.66
dArg	1.13	1.24	1.13	1.13	1.13	1.24
Vitamin C, mg/kg	--	--	94.38	--	--	94.38
Calcium	0.75	0.75	0.75	0.75	0.75	0.75
Non-Phytate Phosphorus	0.38	0.38	0.38	0.38	0.38	0.38
Total Phosphorus	0.49	0.49	0.49	0.49	0.49	0.49
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
	Analyzed Nutrient Content, %					
Fat	6.1	6.2	6.4	6.3	6.4	6.2
Crude fiber	2.8	2.5	3.4	3.2	3.2	3.7
Ash	4.0	4.1	4.1	4.3	4.5	4.3
Dry matter	89.1	89.5	89.4	89.4	89.0	89.5

Table 3. Continued

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[2] Vitamin premix added at this rate yields 15 400 IU vitamin A, 11 000 ICU vitamin D3, 110 IU vitamin E, 3 mg vitamin K-3, 0.02 mg B12, 13.2 mg riboflavin, 77 mg niacin, 19.8 mg d-pantothenic acid, 1.76 mg folic acid, 5.5 mg pyroxidine, 3.08 mg thiamine, 0.16 mg biotin per kg diet

[3] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 12-24). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[4] Rodimet®NP99, Adisseo France SAS.

[5] Ajinomoto Heartland Inc., Eddyville, IA

[6] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[7] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[8] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.

[9] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[10] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[11] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine, Vitamin C and/ or vitamin premix.

[12] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 2 and 6 were adjusted based on the amount of supplemental L-Arg and its level of CP.

Table 4. Withdrawal dietary formulations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (37 to 45 days-of-age)

Ingredient	Control[1]	High Arginine	Vitamin C	2X Vitamin[2]	Reduced AA[3]	Combin-ation[2]
	------------------------------------ % ------------------------------------					
Corn	64.56	64.56	64.56	64.56	64.56	64.56
Soybean Meal	21.39	21.39	21.39	21.39	21.39	21.39
Corn DDGS	8.00	8.00	8.00	8.00	8.00	8.00
Soybean Oil	3.00	3.00	3.00	3.00	3.00	3.00
Meat & Bone Meal	0.93	0.93	0.93	0.93	0.93	0.93
Limestone	0.73	0.73	0.73	0.73	0.73	0.73
Salt, NaCl	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine, 99%[4]	0.23	0.23	0.23	0.23	0.23	0.23
L-Lysine HCl, 78.8%[5]	0.25	0.25	0.25	0.25	0.25	0.25
L-Threonine, 98.0%[5]	0.09	0.09	0.09	0.09	0.09	0.09
L-Valine, 96.5%[5]	0.02	0.02	0.02	0.02	0.02	0.02
Choline-Cl, 60%	0.08	0.08	0.08	0.08	0.08	0.08
Selenium Premix, 500 ppm[6]	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[7]	0.06	0.06	0.06	0.06	0.06	0.06
Vitamin E, 500 IU/g	0.02	0.02	0.02	0.02	0.02	0.02
Phytase[8]	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[5]	--	0.11	--	--	--	0.11
Vitamin C[9]	--	--	0.03	--	--	0.03
Vitamin Premix	0.02	0.02	0.02	0.04	0.02	0.04
Cellulose, Filler (wt: wt)[10]	0.16	0.05	0.13	0.14	0.16	--
Nutrient	Calculated Nutrient Content, %					
AME, kcal/kg	3235	3235	3235	3235	3235	3235
Protein[11]	18.25	18.46	18.25	18.25	18.25	18.46
dLys	0.90	0.90	0.90	0.90	0.90	0.90
dMet	0.48	0.48	0.48	0.48	0.48	0.48
dSAA	0.82	0.82	0.82	0.82	0.82	0.82
dThr	0.61	0.61	0.61	0.61	0.61	0.61
dArg	1.16	1.27	1.16	1.16	1.16	1.27
Vitamin C, mg/kg	--	--	94.38	--	--	94.38
Calcium	0.65	0.65	0.65	0.65	0.65	0.65
Non-Phytate Phosphorus	0.33	0.33	0.33	0.33	0.33	0.33
Total Phosphorus	0.44	0.44	0.44	0.44	0.44	0.44
Sodium	0.19	0.19	0.19	0.19	0.19	0.19
	Analyzed Nutrient Content, %					
Fat	7.6	7.1	6.9	7.1	7.1	7.1
Crude fiber	2.9	2.7	2.8	3.1	3.0	3.3
Ash	4.1	3.9	3.7	3.7	3.9	4.0
Dry matter	89.8	88.7	88.8	89.0	89.8	89.2

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

Table 4. Continued

[2] Vitamin premix added at this rate yields 15 400 IU vitamin A, 11 000 ICU vitamin D_3, 110 IU vitamin E, 3 mg vitamin K-3, 0.02 mg B_{12}, 13.2 mg riboflavin, 77 mg niacin, 19.8 mg d-pantothenic acid, 1.76 mg folic acid, 5.5 mg pyroxidine, 3.08 mg thiamine, 0.16 mg biotin per kg diet

[3] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 12-24). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[4] Rodimet®NP99, Adisseo France SAS.

[5] Ajinomoto Heartland Inc., Eddyville, IA

[6] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[7] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[8] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[9] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[10] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine, Vitamin C and/ or vitamin premix.

[11] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 2 and 6 were adjusted based on the amount of supplemental L-Arg and its level of CP.

Animals and Management Practices

The animal care protocol was developed in accordance with and approved by TX A&M’s Institutional Animal Care and Use Committee (IACUC). On day of hatch, 1,980 high-yielding male broiler chicks were randomly allotted to blocked floor pens and dietary treatments based on initial body weight. The study consisted of a total of 66 pens (1.83m x 1.83m) with each treatment consisting of 11 replicate pens and 30 birds per replicate pen. Pens were equipped with tube feeders (feeder base circumference of 1.0368 m (area = 0.086 m^2), nipple drinkers, and top-dressed recycled litter from 4 previous flocks. Accounting for the feeder space, the stocking density in each pen was 0.109 m^2/bird, which is greater than typical industry standards in the USA (0.083 m^2/bird). Environmental heating and lighting programs were consistent with industry standards: d 0-3, 24h of light at 2-foot candles, d 4-7, 20 h of light at 2-foot candles, d 8-14, 16 h of light at 1-foot candle, d 15-42, 16 h of light at 0.2 food candles, and d 43-45, 23 h of light at 0.2-foot candles.

The 4 phase growout program consisted of a starter that was fed from day-of-hatch to 12 d-of-age, grower from 13-24 d-of-age, finisher from 25-36 d, and withdrawal from d 37-45; *ad libitum* access to feed and water was provided throughout the trial. All broilers and feed were weighed on the day the dietary feed changes were made in order to determine an average body weight (BW), feed consumption (FC), mortality-corrected feed conversion ratio (FCR) and cumulative FCR.

Processing

Upon completion of the trial (d 45) broilers within each pen were weighed. Seven broilers were selected for processing from each replicate pen (+/- 300 grams of the pen mean; 462 birds in total based on 6 treatments; 11 replicates/ treatment; 7 birds/replicate). These birds were then placed on an 8-hour feed withdrawal period prior to processing on d 46. All birds were conventionally processed in a pilot scale processing facility at Texas A&M University. Birds were stunned (Model SF-7000, Simmons Engineering Corp., Dallas, GA) in a 1% saline bath, 13 mA, 7 s, 500 Hz, DC and bled using a unilateral neck cut. The exsanguinated birds were allowed to bleed for 90 sec. All birds were conventionally scalded (61 C, 45 sec), picked in a rotary drum picker (Model sp30ss, Bower Corp., Houghton, LA 52631) for 25 sec, and manually eviscerated. Birds were then chilled to 4 C within 80 minutes. The following parameters were measured on the day of processing: fasted live weight, hot carcass weight

(WOG_{HC}), chilled carcass weight (WOG_{CC}) and the weight of cut-up parts (skinless boneless breast (*Pectoralis major*), tenders (*Pectoralis minor*), wings, and leg quarters (thigh + drumstick). After weighing, the left and right *Pectoralis major* filets were palpated and scored for WS based on the scoring system of Kuttappan et al. (2012) and WB based on the scoring system of Tijare et al. (2016).

Statistical Analysis

All data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model Procedure (SPSS V18); pen was used as the experimental unit. Percentage and categorical data (mortality, processing yields, and WS and WB incidence data) were arc-sine transformed for analysis. Means that were significantly different at $p<0.05$ were separated using Duncan's Multiple Range Test.

Results

Performance

Growth and FCR of the broilers in Treatment 1 were in line with performance guidelines for the high-yielding broiler strain that was used. Following the starter phase (1-12 d-of-age), no statistical differences in BW ($p = 0.232$; Table 5), FC ($p = 0.117$; Table 6) or mortality-corrected FCR ($p = 0.066$; Table 7) were observed.

In the grower phase (13 to 24 d-of-age), reducing the amino acid density by 15% in Treatments 5 and 6 led to significantly lower BW compared to the first four treatments ($p < 0.001$); however, there was no statistical difference in the BW between Treatments 1 through 4. Even though there were no differences in feed consumption between treatments in the grower phase ($p = 0.379$), FCR was statistically greater for

Treatments 5 and 6 and lowest for Treatments 1 through 4 ($p < 0.001$); this effect was strong enough to affect these treatments across the entire 1 to 24 d-of-age period ($p < 0.001$).

Following the finisher phase (25 to 36 d-of-age), BW was highest for Treatment 4 (2.405 kg/bird; $p < 0.001$), lowest for Treatment 6 (2.267 kg/bird; $p < 0.001$), and similar between Treatments 1, 2, 3, and 4 ($p > 0.05$). No statistical differences in BW were observed between Treatments 5 and 6 or between Treatments 1 and 5 ($p > 0.05$). While this pattern reflects the dLys intake for this period ($p = 0.05$), the dLys intake for the 1 to 36 d period was lowest for Treatments 5 and 6 ($p < 0.001$). Among the treatments, FC was lowest for Treatment 6 (1.831 kg/bird; $p = 0.001$), highest for Treatment 4 (1.929 kg/bird; $p = 0.001$), and there was no difference in FC between Treatments 1, 2, 3 and 5 ($p > 0.05$). Unlike in the starter and grower phases, there was no difference in FCR amongst the 6 treatments ($p = 0.183$) during the finisher phase. For the 1 to 36 d-of-age period, however, FCR was greatest for treatments 5 and 6, and lowest for Treatments 2 through 4 ($p < 0.001$).

At the end of the withdraw phase (37 to 45 d-of-age) and cumulatively throughout the growout (1 to 45 days-of-age), all dietary treatments had similar BW ($p = 0.220$ and $p = 0.675$, respectively) and FC ($p = 0.482$ and $p = 0.202$, respectively). In this phase, there was no difference in dLys intake ($p = 0.483$); however, there was a significant difference for total dLys consumption over the 1 to 45 day period, whereby, Treatments 5 and 6 had the lowest dLys intake ($p < 0.001$). Differences in mortality-corrected FCR were observed for the withdraw phase ($p < 0.001$) and the complete 1-45 day growout (p < 0.001). In the 37 to 45 d period, the mortality-corrected FCR was greatest for Treatments 2, 3, 4 (p < 0.001) and lowest for Treatments 1, 5 and 6 (p = 0.05). For the 1 to 45 d growout, the mortality-corrected FCR was highest for Treatments 3 and 4, lowest for Treatment 5, and similar between Treatments 1, 2 and 6 (p = 0.05). Adjusting the mortality-corrected FCR to a common BW, the FCR was greatest for Treatments 3, 4 and 6 and lowest for Treatments 1, 2 and 5 (p = 0.006). No differences were observed in mortality in any of the phases or cumulatively for the entire growout period.

Table 5. Body weight and mortality of male broilers fed diet with a higher ratio of dArg:dLys vitamin premix, vitamin C and/ or a lower digestible amino acid ratio

	d 12	d 24	d 36	d 45	d 1-45
Treatment	g/bird	------------------------ kg/bird ------------------------			
Control	339.2	1.182[a]	2.339[bc]	3.074	5.8
High Arginine[1]	342.2	1.195[a]	2.376[ab]	3.094	3.0
Vitamin C[2]	341.2	1.195[a]	2.362[ab]	2.973	4.2
2X Vitamin[3]	341.5	1.204[a]	2.405[a]	3.017	6.4
Reduced AA[4]	339.2	1.126[b]	2.284[cd]	3.037	4.9
Combination[5]	346.9	1.121[b]	2.267[d]	2.983	4.2
PSEM	1.06	0.0068	0.0108	0.0561	2.90
P-value	*0.232*	*<0.001*	*<0.001*	*0.220*	*0.675*

a-d Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The dig Arg:dig Lys ratio was increased from 112 to 1.20 in the starter, 114 to 126 in the grower, 115 to 126 in the finisher, and 114 to 126 in the withdraw phase.

[2] The level of dietary vitamin C was 94.38 ppm.

[3] An increase in the vitamin premix concentration (2X).

[4] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[5] Birds were fed, the starter, finisher and withdraw from Treatment 1, and the grower from Treatment 5; however, all were supplemented with dietary arginine, Vitamin C, and the 2x vitamin premix.

Table 6. Feed consumption of male broilers fed diets with a higher ratio of dArg: dLys, vitamin premix, vitamin C and/ or a lower digestible amino acid ratio

Treatment	Starter	Grower	Finisher	WD	Day 1-24	Day 1-36	Day 1-45
	-- kg/bird --						
	Starter	Grower	Finisher	WS	Day 1-24	Day 1-36	Day 1-45
Control	0.350	1.153	1.899[ab]	1.664	1.504	3.404[ab]	5.069
High Arginine[1]	0.351	1.161	1.906[ab]	1.673	1.512	3.418[ab]	5.092
Vitamin C[2]	0.344	1.184	1.900[ab]	1.648	1.528	3.429[ab]	5.077
2X Vitamin[3]	0.356	1.197	1.929[a]	1.648	1.553	3.483[a]	5.131
Reduced AA[4]	---	1.168	1.868[bc]	1.671	1.516	3.385[b]	5.057
Combination[5]	0.358	1.164	1.831[c]	1.603	1.522	3.354[b]	4.957
PSEM	1.599	6.440	9.346	14.451	7.130	14.032	25.997
P-value	0.117	0.379	0.001	0.482	0.470	0.035	0.202

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The dig Arg:dig Lys ratio was increased from 112 to 1.20 in the starter, 114 to 126 in the grower, 115 to 126 in the finisher, and 114 to 126 in the withdraw phase.

[2] The level of dietary vitamin C was 94.38 ppm.

[3] An increase in the vitamin premix concentration (2X).

[4] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[5] Birds were fed, the starter, finisher and withdraw from Treatment 1, and the grower from Treatment 5; however, all were supplemented with dietary arginine, Vitamin C, and the 2x vitamin premix.

Table 7. Feed conversion of male broilers fed diets with higher ratio of dArg: dLys, vitamin premix, vitamin C and/ or a lower digestible amino acid ratio

Treatment	Starter	Grower	Finisher	WD	Day 1-24	Day 1-36	Total d 1-45	Wt. Corrected FCR, d 1-45[1]
Control	1.189	1.367[b]	1.636	2.222[cd]	1.325[b]	1.481[b]	1.660[bc]	1.637[b]
High Arginine[2]	1.182	1.357[b]	1.609	2.357[c]	1.311[b]	1.461[c]	1.663[bc]	1.634[b]
Vitamin C[3]	1.161	1.387[b]	1.620	2.656[b]	1.325[b]	1.473[bc]	1.708[a]	1.716[a]
2X Vitamin[4]	1.203	1.372[b]	1.602	2.932[a]	1.327[b]	1.463[c]	1.712[a]	1.706[a]
Reduced AA[5]	-----	1.488[a]	1.611	2.129[d]	1.400[a]	1.508[a]	1.653[c]	1.622[b]
Combination[6]	1.189	1.502[a]	1.596	2.256[cd]	1.412[a]	1.507[a]	1.692[ab]	1.697[a]
Table 7. Continued								
PSEM	0.0042	0.0084	0.0049	0.0709	0.0058	0.0034	0.0113	0.0283
P-value	*0.066*	*<0.001*	*0.183*	*<0.001*	*<0.001*	*<0.001*	*0.001*	*0.006*

a-d Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] Weight corrected FCR is corrected so that 1 point of FCR is equal to 32g of body weight.

[2] The dig Arg:dig Lys ratio was increased from 112 to 1.20 in the starter, 114 to 126 in the grower, 115 to 126 in the finisher, and 114 to 126 in the withdraw phase.

[3] The level of dietary vitamin C was 94.38 ppm.

[4] An increase in the vitamin premix concentration (2X).

[5] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[6] Birds were fed, the starter, finisher and withdraw from Treatment 1, and the grower from Treatment 5; however, all were supplemented with dietary arginine, Vitamin C, and the 2x vitamin premix.

Table 8. Digestible lysine consumption of male broilers fed diets with a higher ratio of dArg: dLys, vitamin premix, vitamin C and/ or a lower digestible amino acid ratio

Treatment	Starter	Grower	Finisher	WD	Day 1-24	Day 1-36	Day 1-45
	g/bird						
Control	4.21	12.58[a]	18.62[ab]	14.98	16.79[a]	35.41[a]	50.39[a]
High Arginine[1]	4.22	12.66[a]	18.68[ab]	15.06	16.87[a]	35.56[a]	50.62[a]
Vitamin C[2]	4.13	12.92[a]	18.62[ab]	14.84	17.04[a]	35.67[a]	50.50[a]
2X Vitamin[3]	4.26	13.06[a]	18.91[a]	14.84	17.32[a]	36.23[a]	51.07[a]
Reduced AA[4]	----	10.82[b]	18.31[bc]	15.05	14.99[b]	33.31[b]	48.36[b]
Combination[5]	4.29	10.78[b]	17.95[c]	14.43	15.07[b]	33.03[b]	47.46[b]
PSEM	0.019	0.135	0.092	0.130	0.137	0.202	0.290
P-value	*0.179*	*<0.001*	*0.001*	*0.483*	*<0.001*	*<0.001*	*<0.001*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The dig Arg:dig Lys ratio was increased from 112 to 1.20 in the starter, 114 to 126 in the grower, 115 to 126 in the finisher, and 114 to 126 in the withdraw phase.

[2] The level of dietary vitamin C was 94.38 ppm.

[3] An increase in the vitamin premix concentration (2X).

[4] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[5] Birds were fed, the starter, finisher and withdraw from Treatment 1, and the grower from Treatment 5; however, all were supplemented with dietary arginine, Vitamin C, and the 2x vitamin premix.

Processing

No differences were observed across the treatments with regard to processing weights of the live, fasted broiler, carcass, or any parts (Table 9). Because no differences were observed in live weight or dLys of Treatments 1 through 4 at 45 d-of-age, this was somewhat expected; however, it was not anticipated for treatments 5 and 6 given the lower dLys intake. As a percentage of live, fasted weight, no differences were observed in carcass or parts yield between any of the treatments, with the exception of breast meat yield. While all of other dietary treatments were similar to the control diet, including the diet with a reduction in AA density during the grower phase, supplementing vitamin C into the formulation either individually or in the combination treatment reduced breast meat yield ($p < 0.001$).

Table 9. Processing weights and processing yields of male broilers fed diets with a higher ratio of dArg: dLys, vitamin premix, vitamin C and/ or a lower digestible amino acid ratio

	Processing Weights						Processing Yields, %				
Treatment	Live, Fasted, kg	Chilled carcass, kg	Breast, g	Tender, g	Leg, g	Wing, g	Chilled Carcass	Breast	Tender	Leg	Wing
Control	2.993	2.376	712.9	129.4	726.7	253.3	79.52	29.89[a]	5.39	30.58	10.66
High Arginine[1]	3.057	2.433	712.5	135.0	751.7	256.9	79.59	29.26[abc]	5.55	30.90	10.58
Vitamin C[2]	2.922	2.321	676.3	125.0	707.8	246.1	79.41	29.17[bc]	5.40	30.52	10.64
2X Vitamin[3]	2.988	2.374	696.1	129.3	730.6	254.3	79.47	29.28[abc]	5.45	30.81	10.56
Reduced AA[4]	2.980	2.390	711.5	127.6	717.7	253.7	79.03	29.74[ab]	5.43	30.46	10.64
Combination[5]	2.936	2.327	674.4	126.5	716.7	248.1	79.25	28.92[c]	5.43	30.85	10.69
PSEM	0.026	0.019	0.008	0.001	0.006	0.002	0.09	0.11	0.087	0.070	0.04
P-value	*0.393*	*0.306*	*0.188*	*0.087*	*0.087*	*0.561*	*0.624*	*0.046*	*0.706*	*0.192*	*0.957*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The dig Arg:dig Lys ratio was increased from 112 to 1.20 in the starter, 114 to 126 in the grower, 115 to 126 in the finisher, and 114 to 126 in the withdraw phase.

[2] The level of dietary vitamin C was 94.38 ppm.

[3] An increase in the vitamin premix concentration (2X).

[4] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[5] Birds were fed, the starter, finisher and withdraw from Treatment 1, and the grower from Treatment 5; however, all were supplemented with dietary arginine, Vitamin C, and the 2x vitamin premix.

White Striping and Woody Breast

Treatments effects were not significant for WS (p = 0.548) at the termination of the trial (Table 10). Compared to the control-fed birds (Treatment 1), the average woody breast score was significantly reduced (p < 0.001) by each of the feed additives (Treatments 2-4), the reduction in dLys during the grower phase, (Treatment 5) and their combination (Treatment 6; Table 10). Ranking the order of effectiveness at reducing the average WB score, feeding Treatment 3 resulted in the lowest score (1.25; vitamin C), followed by Treatments 6 (1.42; combination), 2 (1.49; L-Arginine), 5 (1.53; low dAA grower), 4 (1.75; 2x vitamin premix) and 1 (1.84; control). Reviewing incidence of WB scores for each treatment, it is apparent that there was a significant downward shift (i.e. an improvement) in the incidence of #3 scores (p = 0.033) and an improvement in the #0 scores (p = 0.083). This shift in score is most pronounced when the cumulative incidence of 0&1 (most desired) vs. 2&3 (least desired) scores are compared. Compared to the control diet that had a 2&3 incidence score of 61.3%, the respective incidence of 2&3 scores for Treatments 2, 3, 4, 5 and 6 dropped by 19.62, 41.48, 2.12, 17.10 and 36.87%. Comparing the 0&1 incidence of the control-fed birds (38.70%), the incidence of 0&1 scores amongst Treatments 2, 3, 4,5 and 6 increased by 31.09, 65.68, 3.36, 27.08 and 58.40%, respectively. Just as for the mean scores, Treatment 1 had the highest and Treatment 3 had the lowest incidence of 2&3 scores (p = 0.048); the converse was observed for the 0&1 scores (p = 0.048). While the inclusion of vitamin C (Treatment 3) reduced the average WB score by 32% and improved the incidence of 0&1 scores by 65.68%, a significant reduction in breast meat yield was unexpectedly observed.

Table 10. Meat quality measurements and woody breast profile of male broilers that are fed diets with a higher ratio of dArg: dLys, vitamin premix, vitamin C and/ or a lower digestible amino acid ratio

	Meat Quality Measurements		Woody Breast Profile					
Treatment	Ave. Woody Breast Score	Ave. White Striping Score	Score of 0, %	Score of 1, %	Score of 2, %	Score of 3, %	Score of 0&1, %	Score of 2&3, %
Control	1.84[a]	1.29	2.81	35.90	37.20	24.4[a]	38.70[c]	61.30[a]
High Arginine[1]	1.49[bcd]	1.19	7.90	42.91	41.64	7.73[b]	50.73[abc]	49.27[abc]
Vitamin C[2]	1.25[d]	1.09	14.50	50.00	32.38	3.50[b]	64.12[a]	35.87[c]
2X Vitamin[3]	1.75[ab]	1.30	2.81	37.10	42.90	17.1[ab]	40.00[bc]	60.00[ab]
Reduced AA[4]	1.53[bc]	1.20	13.10	36.45	35.18	15.55[ab]	49.18[abc]	50.82[abc]
Combination[5]	1.42[cd]	1.09	7.16	54.20	27.30	11.40[ab]	61.30[ab]	38.70[bc]
PSEM	0.053	0.031	1.492	2.532	2.592	1.914	3.098	3.098
P-value	*<0.001*	*0.548*	*0.083*	*0.126*	*0.451*	*0.033*	*0.048*	*0.048*

a-d Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The dig Arg:dig Lys ratio was increased from 112 to 1.20 in the starter, 114 to 126 in the grower, 115 to 126 in the finisher, and 114 to 126 in the withdraw phase.

[2] The level of dietary vitamin C was 94.38 ppm.

[3] An increase in the vitamin premix concentration (2X).

[4] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[5] Birds were fed, the starter, finisher and withdraw from Treatment 1, and the grower from Treatment 5; however, all were supplemented with dietary arginine, Vitamin C, and the 2x vitamin premix.

Discussion

Woody breast and white striping are described very well by Petracci and Cavani (2012), Kuttappan et al. (2013) and Shivo et al. (2014). Across these studies, the two myopathies / conditions are characteristically expressed by modern, fast-growing and high-yielding broiler strains that are grown over a long period of time, to a heavy weight classes (ex. 56 to 63 days at 3.63 to 4.76 kg/bird). While the incidence and severity of WS and WB are certainly age related, previous research conducted by Texas A&M University was able to elicit a >95% incidence in WB for high-yielding male broilers at 49 days-of-age when high, nutrient-dense diets were fed; broilers had an average body weight of 3.77 kg/bird (average daily gain = 89.8 g/day) and a feed conversion ratio (FCR) of 1.60 (unpublished data). Regardless of the incidence and severity, the previously mentioned authors observed that breast tissue with WB foci exhibit polyphasic myodegeneration and regeneration, variable amounts and types of muscle and connective tissue, perivenular lymphocytic accumulation, and variable levels of vasculature thickening and damage. In addition, to the physical changes within the tissue, there is often a characteristic accumulation of a serous extracellular exudate that lies between the skin and the breast tissue.

Defining the etiology of WS and WB, several authors have studied whether WS and WB are heritable (i.e. genetics), nutritional, environmental or metabolic in nature. The fact today is that no one study has unequivocally determined what causes the two myopathies. While the WS and WB are not a food safety issue, the visible and textural differences of each are different enough from “normal” that 1. Customers (i.e. grocery and restaurant chains) and consumers (i.e. anyone that physically purchases and eats the prepared product or cut of meat) have started to recognize WS and WB, 2. Recognition of the difference between effected vs non- effected meat, and 3. Demanding changes in how broilers are raised for meat.

This study sought to define nutritional strategies that might lead to the elimination or a significant reduction in one or both conditions. This was accomplished by using feed additives or levels of nutrients that are known to have nutritional and physiological properties. The issues today are helping to define the etiology and solutions for the two muscle myopathies. In order to understand these myopathies, the cellular and muscular structure must be understood. Skeletal muscle cell types are divided into two categories 1. Glycolytic (white fiber, rapid twitch) and 2. Aerobic/ oxidative (red fiber, slow twitch). Regardless of cell type, the hierarchical structure of muscle tissue is based on satellite cells that develop *in ovo* and early on post-hatch (Sobolewska et al., 2011; Ordahl et al., 1999; Harthan et al., 2013).

Satellite cells are essential to muscle development and repair due to the ability to be the "stem cells" that give rise to mono-nucleated myoblasts through proliferation, differentiation and fusion with adjacent muscle fibers (Moss and Leblond, 1971). The degree to which these processes take place depends on the concentration and location of satellite cells, and the fiber type. While white, fast-twitching, glycolytic muscle fibers contain few satellite cells, the slow-twitch, oxidative muscle fibers contain many. In the *Pectoralis major* of broiler chickens (fast twitch, glycolytic), satellite cells are found in the greatest concentration at the peripheral edge of the growing breast tissue, while they are more numerous and diffuse throughout the slow-twitch, oxidative muscle fibers (ex. *Sartorius major*). In both muscle fiber types, regeneration is an ongoing process that occurs when new fibers are produced during postnatal growth and further regenerated after injury and/or death of muscle (Gerrad and Grant, 2003; Mann et al., 2011; Harthan et al., 2013). Compounding the cellular hierarchy and rapid, continuous and simultaneous rates of protein turnover (i.e. accretion vs. degradation) that occurs within the muscle cells (Goll et al., 1992; Gerrad and Grant, 2003), the breast tissue (*P. majors* and *P. minors*) is tightly compartmentalized against the keel and is poorly vascularized when compared to red muscle types (ex. *Sartorius major)* (Bilgili, 2013). Due to the difference in number and location of the satellite cell populations, the amount of vasculature within each of the muscle types, and the continuous remodeling of the breast tissue on a macro scale, the response time and degree of tissue repair often occurs at disparate rates.

In this study, multiple nutritional strategies were investigated in an effort to mitigate the presence and severity of WS and WB, without impacting performance. As one of five nutritional strategies, L-Arginine was selected as a potential mitigating strategy, as Bautista-Ortega and Ruiz-Feria (2010) demonstrated that feeding higher levels of Arginine caused vasodilation via an increase in nitric oxide production. As a result of this dilation, it is theorized that efficiency of nutrient and metabolite exchange in the poorly vascularized breast tissue can be improved (Jobgen et al., 2006). The enhanced nutrient usage is also crucial in the hypertrophy of the muscle tissue (Fernades et al., 2009).

Pulmonary hypertension syndrome, a metabolic disorder that commonly affects fast growing broilers and is often found in hypoxic conditions, was observed to be impacted by the inclusion of arginine, vitamin C, and vitamin E (Bautista-Ortega and Ruiz-Feria, 2010). The results from the study indicated that the high arginine dietary treatment and Arg + Vit E + Vit C treatment both showed a significant reduction in pulmonary artery reactivity. Sumou et al. (2006) also reported arginine's ability to reduce hypoxic pulmonary artery remodeling in broilers. These data suggest that arginine improved the flow of oxygen in the blood which in turn had a positive effect on the hypoxic state of the muscle.

Bilgili (2013) hypothesized that localized hypoxia is present in woody breast tissue due to the reduction in the capillary supply. Mutryn et al. (2015) validated the hypothesis when the gene, hypoxia-inducible factor -1(HIF-1), was discovered to activate the transcription factor that is present in birds affected with WB. One of the genes regulated by HIF-1, *PLOD2*, is upregulated in WB-affected birds. This important gene, associated with extracellular stiffening and collagen alignment, can be linked to fibrosis which is a common characterization of WB (Van der Slot, 2003). This upregulation process may also play a role in the changes that occur within in the extracellular matrix which causes stiffness of WB. From these studies, it can be suggested that when hypoxia is present in the muscle and there is an inclusion of arginine, there is an overall reduction of hypoxia; reducing WB severity. The present hypothesis confirmed the previous studies mentioned by also showing the reduction of WB by the use of dietary arginine treatments while also establishing a lack of impact on the overall breast meat yield.

Additionally, supplementing vitamin C has been recommended to reduce the incidence of woody breast in today's modern broilers (Abasht et al., 2016). Vitamin C has also been reported to provide support for anti-oxidative metabolism by eliminating oxygen derived free radicals (Xiang et al., 2002) and for the biosynthesis of collagen (Abasht et al., 2016). Recently, Abasht et al. (2016) hypothesized that the glycogen depletion that is seen in WB affected birds can be contributed to the over activation of the ascorbate (vitamin C) biosynthesis pathway. Abasht et al. (2016) concluded with the recommendation of a vitamin C supplementation to decrease the activity of the pathway and reduce the damage done to the breast tissue.

In a study conducted by Mutryn et al. (2015), WB affected birds were observed to have undergone oxidative stress, caused by an increase in reactive oxygen species (ROS). An increase in ROS is detrimental to muscles due to its ability to change cellular pathways which ultimately results in a change in skeletal muscle remodeling and adaptation (Powers et al., 2010). It was hypothesized that vitamin E and vitamin C may neutralize ROS by reacting with nitric oxide to produce peroxynitirte, in turn sparing nitric oxide and increasing vasodilation (Bautista-Ortega and Ruiz-Feria, 2010). Similarly, vitamin C has also been reported to improve resistance to many metabolic stressors such as hypoxia, which contributes to the production of oxygen-derived free radicals (Agudelo, 1983; Al-Taweil & Kassab, 1990; Bottje and Wideman, 1995). The elimination of oxygen derived free radicals with the use of vitamin C is a possible explanation for the reduction of hypoxia (Xiang et al., 2002). The current study showed, the inclusion of vitamin C reduced the WB score significantly when compared to the control diet perhaps through the elimination of hypoxia.

Furthermore, in the current study, the reduction in amino acidy density in the grower phase was believed to maximize satellite cell development to allow the breast tissue to recover from rapid growth in the starter phase (Powell et al., 2014). Amino acids are known to regulate key metabolic pathways, optimize muscle growth and enhance protein synthesis (Wu, 2009). Previous studies reported the necessity of satellite cell development in the neonatal stage for protein synthesis and muscle development (Powell et al., 2013). Satellite cells were observed by Pophal et al. (2004) to be sensitive to nutrition in their mitotic activity when fed differing levels of lysine. Similarly, Powell et al. (2013) observed the reduction in satellite cell activity when Met/Cys were reduced in the diet. Decreasing amino acid density in the current study reduced BW and increased FCR throughout the grower phase; however, compensatory gain resulted in similar growth performance parameters to the control at the conclusion of the experiment. These results are congruent with the study conducted by Kidd et al. (2005) which found broilers fed lower amino acid diets experience a reduction in BW, feed conversion and carcass yield. These studies suggest the lowered AA density reduced BW in the grower phase by decreasing satellite cell activity which allowed for the recovery of the muscle. In conclusion, the current study reduced amino acid density during the grower phase and did not impact final growth performance but did reduce the overall incidence of WB without negatively impacted breast yield similar to the arginine dietary treatment.

These data indicate that the nutritional strategies including an increase in digestible arginine, inclusion of vitamin C. or a reduced AA density diet during the grower phase could be possible solutions to mitigate WB. Arginine improved vasculature in hypoxic conditions which has been found in WB-affected broilers.

Similarly, vitamin C had a positive impact on oxidative stress by eliminating oxygen-derived free radicals. Furthermore, reduced AA density reduced satellite cell development in the grower phase for the recovery of breast tissue. Subsequent studies need to be conducted on differing inclusion rates of arginine and vitamin C to determine the maximum beneficial level. Additionally, subsequent studies should evaluate multiple time period of AA density reduction to determine the most appropriate time to utilize this strategy.

CHAPTER III

THE EVALUATION OF VARYING DOSES OF DIETARY ARGININE AND/ OR VITAMIN C ON BROILER PERFORMANCE, MEAT YIELD AND THE INCIDENCE OF WHITE STRIPING AND WOODY BREAST

Introduction

The United States is currently the largest broiler industry in the world, producing over 40 million pounds of broiler meat every year, with 19 percent of exports sent to Mexico, Canada, and Hong Kong. Since 1960, poultry per capita consumption in the United States has quadrupled to roughly 108 pounds per person. This can be attributed to health benefits, convenience, and fair prices that poultry protein offers (National Chicken Council, 2016). The demand for poultry continues to rise and thus total production must increase. The industry has responded by increasing the genetic potential for growth rate, continued evaluation of nutrition to maximize meat yield, and improving management practices to allow for higher livability and growth rate. These advancements have led to a higher yielding broiler that is produced in less time than historically observed. In 2016, a broiler that weighs 2.8 kg was able to be produced with 5.2 kg of feed. This is a 3.30% year over year increase in body weight and a 2.55% reduction in FCR year over year (National Chicken Council, 2016).

However, with this improvement in performance, it has become evident that some muscle myopathies may be associated with the increase in growth rate (Kuttappan et al., 2012; Russo et al., 2015; Trocino et al., 2015). These myopathies have been related to an increase in metabolic stress (Hoving-Bolink et al., 2000; Macrae et al., 2006), an increase in muscle damage (Velleman and Nestor, 2003), and the incidence of pale, soft, exudative meat (Wang et al., 1999) in faster growing broilers. Petracci et al. (2015) stated that various meat quality defects could result from the reduced glycolytic potential and thermoregulatory capacity in modern fast growing broilers.

Two muscle myopathies that have been associated with fast growth rate and have garnered major attention in recent years, is white striping (WS) and woody breast (WB). The incidence of these myopathies is being reported globally including Brazil, Italy, Finland, United States, and the United Kingdom (Kuttappan et al., 2012b; Petracci et al., 2013; Ferreira et al., 2014; Sihvo et al., 2014; de Brot et al., 2016; Kuttappan et al., 2016). White striping is commonly characterized by white striations composed of intramuscular deposits that run parallel to the muscle fiber (Kuttappan et al., 2013). It can be found mainly in the breasts, tenders, and thigh muscles (Kuttappan et al., 2013). However, the severity of WS can vary and the intramuscular deposits will widen in diameter throughout the muscle tissue as the severity increases. Petracci et al. (2014) reported that white striping results in higher fat content and lower protein content, ultimately leading to a higher caloric value meat product. Furthermore, WS has been reported to negatively impact meat quality by increasing cook losses and reducing marinade uptake (Petracci et al., 2013; Petracci et al., 2014; Tijare et al., 2016). The accumulation of these factors has negatively impacted consumer acceptability of product exhibiting high level of WS. A study conducted by Kuttappan et al. (2012a) reported that over 50% of consumers stated they would not purchase a “moderate” to “severe” WS effected breast fillet. This loss in consumer acceptability and perception could potentially lead to tremendous revenue losses (Gee, 2016).

Similar to WS, WB is reportedly linked to fast growth rate and has been observed to possess similar lesions as white striping such as myodegeneration, lipidosis, necrosis, and fibrosis (Kuttappan et al., 2013; Sihvo et al., 2014; Trocino et al., 2015; de Brot et al., 2016). However, WB is uniquely characterized by its pale complexion and rigid breast fillets. Similar to WS, WB can also vary in degrees of severity with the most severe cases resulting in a ridge like bulge extending from the cranial region into the caudal region of the breast fillet (Sihvo et al., 2014). Often times, a clear fluid covering the surface of the breast fillet can be observed. Additionally, WB has also been reported to negatively impact meat quality in the same manner as WS by reducing the marinade uptake and increasing cook loss (Sihvo et al., 2014).

While the etiology of these muscle myopathies is currently unknown, several speculations have emerged on the potential mechanisms responsible. Mutryn et al. (2015) speculated that localized hypoxia, oxidative stress, and muscle fiber type switching, which are commonly associated with faster growing broilers, could influence the presence of these muscle myopathies. Other speculations have also emerged such as age, high nutrient density diets (energy and protein), and genetics (Kuttappan et al., 2012b; Bauermeister et al., 2009; Kuttappan et al., 2015; Kuttapan et al., 2013; Petracci et al., 2013; Lorenzi et al., 2014). However, none of these have been reported to be the definitive cause responsible for these myopathies. Utilizing knowledge of previous studies, physiology, and nutrition, the goal of this study was to evaluate various nutritional strategies that may further reduce or eliminate WS and WB, without negatively impacting growth performance. The nutritional strategies investigated included the supplementation of arginine, vitamin C, and the reduction in amino acid density during the grower phase. Arginine aids in improving blood blow within the muscle and may potentially reduce the hypoxic state of the muscle in WB and WS. Inclusion of vitamin C may improve oxidative stress within the muscle and reduce the incidence and severity of WB. Lastly, the reduction in amino acid density during the grower phase may allow satellite cells to recover from the rapid growth period experienced during the starter phase.

Materials and Methods

Experimental Setup and Dietary Treatments

To evaluate the multiple dietary strategies that may potentially mitigate the incidence and severity of WB and WS, seven treatments were conceived and evaluated using a randomized block design.

Relative to a standard set of commercial broiler diets that were fed until 50 days-of-age in a four-phase growout (Treatment 1; starter, grower, finisher, and withdraw), the strategies investigated were: Reducing the digestible amino acid density in only the grower phase by 15% (include days of age), and feeding the exact same starter, finisher, and withdraw diets that were fed to control (Treatment 2-7), Increasing the level of digestible Arginine: digestible Lysine (dArg:dLys) from ~112% to a minimum of 124% (Treatment 3) or a minimum of 136% (Treatment 4), Supplementing vitamin C at

94.4mg vitamin C/ kg feed (100 ppm on a product basis (Treatment 5)or at 188.8 vitamin C/ kg feed (200 ppm on a product basis; Treatment 6), or Combining vitamin C at 100 ppm and dArg: dLys level at 124% (Treatment 7).

Prior to formulating and mixing the feeds (Tables 11-14), ingredients (corn, SBM, DDGS, MBM) were all analyzed for apparent metabolizable energy (AME), total and digestible amino acids, proximates (crude protein, fat, fiber, moisture, and ash), and both total and phytate phosphorus using Adisseo's NIR platform, PNE (Precise Nutrition Evaluation). After mixing, the treatment feeds were conditioned and pelleted at 70°C and fed *ad libitum* as crumbles in the starter and pellets in the grower, finisher, and withdraw phases.

Table 11. Starter dietary formulations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (1 to 15 days-of-age)

Ingredient	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100ppm Vitamin C	200ppm Vitamin C	Combin-ation[2]
	--------------------------------------- % ---------------------------------------						
Corn	53.68	53.68	53.68	53.68	53.68	53.68	53.68
Soybean Meal	35.85	35.85	35.85	35.85	35.85	35.85	35.85
Corn DDGS	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Soybean Oil	3.15	3.15	3.15	3.15	3.15	3.15	3.15
Limestone	1.19	1.19	1.19	1.19	1.19	1.19	1.19
Salt, NaCl	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Monocalcium Phosphate	0.85	0.85	0.85	0.85	0.85	0.85	0.85
DL-Methionine, 99%[3]	0.34	0.34	0.34	0.34	0.34	0.34	0.34
L-Lysine HCl, 78.8%[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Threonine, 98.0%[4]	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Choline-Cl, 60%	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Selenium Premix, 500 ppm[5]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[6]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[7]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin Premix	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin E, 500 IU/g	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[8]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[4]	--	--	0.15	0.30	--	--	0.15
Vitamin C[9]	--	--	--	--	0.03	0.06	0.03
Cellulose, Filler (wt: wt)[10]	0.30	0.30	0.15	--	0.27	0.24	0.12
Nutrient	Calculated Nutrient Content, %						
AME, kcal/kg	3025	3025	3025	3025	3025	3025	3025
Protein[11]	22.59	22.59	22.59	22.59	22.59	22.59	22.59
dLys	1.20	1.20	1.20	1.20	1.20	1.20	1.20
dMet	0.62	0.62	0.62	0.62	0.62	0.62	0.62
dSAA	0.90	0.90	0.90	0.90	0.90	0.90	0.90
dThr	0.78	0.78	0.78	0.78	0.78	0.78	0.78
dArg	1.35	1.35	1.49	1.63	1.35	1.35	1.49

Table 11. Continued

	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100 ppm Vitamin C	200 ppm Vitamin C	Combin-ation[2]
Vitamin C, mg/kg	--	---	--	--	94.38	188.76	94.38
Calcium	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Non-Phytate Phosphorus	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Total Phosphorus	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Sodium	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Nutrient	Analyzed Nutrient Content, %						
Fat	5.2	5.9	6.0	5.0	6.0	5.6	6.9
Crude fiber	3.1	2.8	2.8	3.6	2.8	2.9	2.8
Ash	6.1	5.6	5.4	5.3	5.3	5.5	5.7
Dry matter	88.3	88.6	87.9	87.9	88.6	88.7	88.9

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[2] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 2,3,4,5, and 7 was reduced by 15% during the grower phase (d 16-29). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[3] Rodimet®NP99, Adisseo France SAS.

[4] Ajinomoto Heartland Inc., Eddyville, IA

[5] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[6] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[7] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.*

[8] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[9] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[10] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine and/or Vitamin C.

[11] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 3, 4, and 7 were adjusted based on the amount of supplemental L-Arg and its level of CP.

Table 12. Grower dietary forumlations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (16 to 29 days-of-age)

Ingredient	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100 ppm Vitamin C	200ppm Vitamin C	Combin-ation[2]
	----------	----------	----------	%	----------	----------	----------
Corn	57.77	65.40	65.40	65.40	65.40	65.40	65.40
Soybean Meal	29.22	22.26	22.26	22.26	22.26	22.26	22.26
Corn DDGS	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean Oil	2.65	1.67	1.67	1.67	1.67	1.67	1.67
Limestone	0.81	0.87	0.87	0.87	0.87	0.87	0.87
Salt, NaCl	0.30	0.29	0.29	0.29	0.29	0.29	0.29
Monocalcium Phosphate	0.16	0.21	0.21	0.21	0.21	0.21	0.21
DL-Methionine, 99%[3]	0.30	0.23	0.23	0.23	0.23	0.23	0.23
L-Lysine HCl, 78.8%[4]	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Threonine, 98.0%[4]	0.09	0.08	0.08	0.08	0.08	0.08	0.08
Choline-Cl, 60%	0.07	0.11	0.11	0.11	0.11	0.11	0.11
Selenium Premix, 500 ppm[5]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[6]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[7]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin Premix	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin E, 500 IU/g	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[8]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[4]	--	--	0.12	0.24	--	--	--
Vitamin C[9]	--	--	--	--	0.03	0.06	0.03
Cellulose, Filler (wt: wt)[10]	--	0.24	0.12	--	0.21	0.18	0.09
	Calculated Nutrient Content, %						
AME, kcal/kg	3085	3085	3085	3085	3085	3085	3085
Protein[11]	20.88	18.15	18.37	18.59	18.15	18.15	18.37
dLys	1.09	0.93	0.93	0.93	0.93	0.93	0.93
dMet	0.57	0.48	0.48	0.48	0.48	0.48	0.48
dSAA	0.83	0.70	0.70	0.70	0.70	0.70	0.70
dThr	0.72	0.61	0.61	0.61	0.61	0.61	0.61
dArg	1.23	1.03	1.15	1.26	1.03	1.03	1.15

Table 12. Continued

	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100 ppm Vitamin C	200 ppm Vitamin C	Combin-Ation[2]
Vitamin C, mg/kg	--	--	--	--	94.38	188.76	94.38
Calcium	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Non-Phytate Phosphorus	0.43	0.43	0.43	0.43	0.43	0.43	0.43
Total Phosphorus	0.52	0.50	0.50	0.50	0.50	0.50	0.50
Sodium	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Nutrient	Analyzed Nutrient Content, %						
Fat	6.4	4.7	5.2	5.9	6.5	5.9	5.5
Crude fiber	3.5	3.0	3.7	4.0	3.0	3.1	2.8
Ash	4.9	4.5	4.4	4.3	4.5	4.7	4.7
Dry matter	89.7	88.7	89.0	89.7	89.4	89.2	89.0

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[2] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 16-29). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[3] Rodimet®NP99, Adisseo France SAS.

[4] Ajinomoto Heartland Inc., Eddyville, IA

[5] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[6] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[7] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.*

[8] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[9] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[10] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine and/or Vitamin C.

[11] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 3,4 and 7 were adjusted based on the amount of supplemental L-Arg and its level of CP.

Table 13. Finisher dietary formulations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (30 to 43 days-of-age)

Ingredient	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100ppm Vitamin C	200ppm VitaminC	Combin-ation[2]
	--------------------------------------- %			---------------------------------------			
Corn	61.81	61.81	61.81	61.81	61.81	61.81	61.81
Soybean Meal	24.99	24.99	24.99	24.99	24.99	24.99	24.99
Corn DDGS	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean Oil	3.11	3.11	3.11	3.11	3.11	3.11	3.11
Limestone	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Salt, NaCl	0.28	0.28	0.28	0.28	0.28	0.28	0.28
DL-Methionine, 99%[3]	0.26	0.26	0.26	0.26	0.26	0.26	0.26
L-Lysine HCl, 78.8%[4]	0.22	0.22	0.22	0.22	0.22	0.22	0.22
L-Threonine, 98.0%[4]	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Choline-Cl, 60%	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Selenium Premix, 500 ppm[5]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[6]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[7]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin Premix	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin E, 500 IU/g	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[8]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[4]	--	--	0.12	0.24	--	--	0.12
Vitamin C[9]	--	--	--	--	0.03	0.06	0.03
Cellulose, Filler (wt: wt)[10]	0.24	0.24	0.12	--	0.21	0.18	0.09
Nutrient			Calculated Nutrient Content, %				
AME, kcal/kg	3155	3155	3155	3155	3155	3155	3155
Protein[11]	19.07	19.07	19.30	19.52	19.07	19.07	19.30
dLys	0.98	0.98	0.98	0.98	0.98	0.98	0.98
dMet	0.55	0.55	0.55	0.55	0.55	0.55	0.55
dSAA	0.76	0.76	0.76	0.76	0.76	0.76	0.76
dThr	0.66	0.66	0.66	0.66	0.66	0.66	0.66
dArg	1.10	1.10	1.22	1.33	1.10	1.10	1.22
Vitamin C, mg/kg	--	--	--	--	94.38	188.76	94.38

Table 13. Continued

	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100 ppm Vitamin C	200 Vitamin C	Combin-ation[2]
Calcium	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Non-Phytate Phosphorus	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Total Phosphorus	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Sodium	0.20	0.20	0.20	0.20	0.20	0.20	0.20
	Analyzed Nutrient Content, %						
Fat	6.9	7.7	7.0	7.6	7.3	7.3	7.5
Crude fiber	2.5	2.8	3.3	3.4	3.0	2.7	3.0
Ash	4.4	4.5	4.0	4.1	4.3	4.2	4.3
Dry matter	89.3	89.7	89.8	89.3	89.1	89.0	88.8

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[2] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 16-29). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[3] Rodimet®NP99, Adisseo France SAS.

[4] Ajinomoto Heartland Inc., Eddyville, IA

[5] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[6] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[7] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria brunetti and Eimeria mivati.*

[8] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[9] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[10] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine and/or Vitamin C.

[11] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 3, 4 and 7 were adjusted based on the amount of supplemental L-Arg and its level of CP.

Table 14. Withdraw dietary formulations, calculated nutrient content, and analyzed nutrient content of treatment diets fed to male broilers (44 to 50 days-of-age)

Ingredient	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100ppm Vitamin C	200ppm VitaminC	Combin-ation[2]
	---------------------------------------	%	---------------------------------------				
Corn	64.70	64.70	64.70	64.70	64.70	64.70	64.70
Soybean Meal	21.95	21.95	21.95	21.95	21.95	21.95	21.95
Corn DDGS	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Soybean Oil	3.42	3.42	3.42	3.42	3.42	3.42	3.42
Limestone	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Salt, NaCl	0.26	0.26	0.26	0.26	0.26	0.26	0.26
DL-Methionine, 99%[3]	0.24	0.24	0.24	0.24	0.24	0.24	0.24
L-Lysine HCl, 78.8%[4]	0.22	0.22	0.22	0.22	0.22	0.22	0.22
L-Threonine, 98.0%[4]	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Choline-Cl, 60%	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Selenium Premix, 500 ppm[5]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Trace Mineral Premix, Se Free[6]	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Salinomycin – SaCox[7]	--	--	--	--	--	--	--
Vitamin Premix	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin E, 500 IU/g	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[9]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Arginine, 96.5%[4]	--	--	0.12	0.24	--	--	0.12
Vitamin C[9]	--	--	--	--	0.03	0.06	0.03
Cellulose, Filler (wt: wt)[10]	0.24	0.24	0.12	--	0.21	0.18	0.09
Nutrient	Calculated Nutrient Content, %						
AME, kcal/kg	3205	3205	3205	3205	3205	3205	3205
Protein[11]	17.57	17.57	17.79	18.01	17.57	17.57	17.79
dLys	0.90	0.90	0.90	0.90	0.90	0.90	0.90
dMet	0.48	0.48	0.48	0.48	0.48	0.48	0.48
dSAA	0.70	0.70	0.70	0.70	0.70	0.70	0.70
dThr	0.61	0.61	0.61	0.61	0.61	0.61	0.61
dArg	0.99	0.99	1.11	1.22	0.99	0.99	1.11
Vitamin C, mg/kg	--	--	--	--	94.38	188.76	94.38

Table 14. Continued

	Control[1]	Reduced AA diet[2]	124 dArg:dLys	136 dArg:dLys	100 ppm Vitamin C	200 ppm Vitamin C	Combin-ation[2]
Calcium	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Non-Phytate Phosphorus	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Total Phosphorus	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Sodium	0.19	0.19	0.19	0.19	0.19	0.19	0.19
	Analyzed Nutrient Content, %						
Fat	7.0	7.6	7.3	7.4	7.5	7.3	7.1
Crude fiber	2.8	2.8	3.0	3.2	3.0	4.2	3.2
Ash	4.8	4.3	3.9	3.9	4.1	3.8	3.9
Dry matter	89.3	89.2	89.1	88.9	89.0	89.4	89.5

[1] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[2] The digestible AA level of all the diets in the starter phase are equal; however, the level of digestible amino acids in treatments 5 and 6 was reduced by 15% during the grower phase (d 16-29). The reduction was made by reducing dLys 15% vs. the Control (Treatment 1) and maintaining the ratio of all other digestible, essential amino acids to dLys.

[3] Rodimet®NP99, Adisseo France SAS.

[4] Ajinomoto Heartland Inc., Eddyville, IA

[5] Selisseo®; R,S-2-Hydroxy-4-methylselenobutanoic acid (HMSeBA). Provides 0.30 ppm of organic selenium. Adisseo France SAS.

[6] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[7] Active drug ingredient salinomycin sodium, 60 g/lb (60 g/lton inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.*

[8] Quantum blue® 5 G, AB Vista Feed Ingredients, Chesterfield, MO.

[9] Rovimix®Stay-C®35 (L-ascorbic acid monophosphate); provides 330,000 mg L-ascorbic acid/kg product. DSM, Elgin, IL.

[10] The level of cellulose (wt: wt) was adjusted based on the amount of L-Arginine and/or Vitamin C.

[11] This source of L-Arginine contains 186% CP, thus the level of dietary CP in Treatments 3, 4, and 7 were adjusted based on the amount of supplemental L-Arg and its level of CP.

Animals and Management Practices

Animal care was provided in accordance with and approved by Texas A&M's Institutional Animal Care and Use Committee (IACUC).

On day of hatch, 2,240 high-yielding male broiler chicks were randomly allotted to blocked floor pens and dietary treatments based on initial body weight. The study consisted of a total of 70 pens (1.67m x 1.83m) with each treatment consisting of 10 replicates pens and 32 birds per replicate pen. Pens were equipped with tube feeders (feeder base circumference of 1.0368 m (area = 0.086 m^2), nipple drinkers, and top-dressed recycled litter from 4 previous flocks. Accounting for feeder space, the stocking density in each pen was 0.093 m^2/bird, which is greater than typical industry standards in the USA (0.083 m^2/bird). Broilers were provided age appropriate supplemental heat and tunnel ventilation. The lighting program was d 0-3, 24 h of light at 2 foot candles, d 4-7, 20 h of light at 2 foot candles, d 8-14, 16 h of light at 1 foot candle, d 15-42, 16 h of light at 0.2 foot candles, and d 43-50, 23 h of light at 0.2 foot candles.

The 4 phase growout program consisted of a starter that was fed from day-of-hatch to 15 d-of-age, grower from 16-29 d-of-age, finisher from 30-43 d, and withdrawal from d 44-50; ad libitum access to feed and water was provided throughout the trial. All broilers and feed were weighed on the day of dietary feed changes for calculation of average body weight (BW), feed consumption (FC), mortality-corrected feed conversion ratio (FCR) and cumulative FCR.

Processing

Upon completion of the trial (d 50) broilers within each pen were weighed. Nine broilers were selected for processing from each replicate pen (+/- 300 grams of the pen mean; 630 birds in total based on 7 treatments; 10 replicates/ treatment; 90 birds/trt). These birds were then placed on an 8 hour feed withdrawal period prior to processing on d 51. All birds were conventionally processed in a pilot scale processing facility at Texas A&M University. Birds were stunned (Model SF-7000, Simmons Engineering Corp., Dallas, GA) in a 1% saline bath, 13 mA, 7 s, 500 Hz, DC and bled using a unilateral neck cut. The exsanguinated birds were allowed to bleed for 90 sec. All birds were conventionally scalded (61 C, 45 sec), picked in a rotary drum picker (Model sp30ss, Bower Corp., Houghton, LA 52631) for 25 sec, and manually eviscerated. Birds were then chilled to 4 C within 80 minutes. The following parameters were measured on the day of processing: fasted live weight, hot carcass weight (WOG_{HC}), chilled carcass weight (WOG_{CC}) and the weight of cut-up parts (skinless boneless breast (*Pectoralis major*), tenderloin (*Pectoralis minor*), wings, and leg (thigh + drumstick). After weighing, the left and right *Pectoralis major* filets were visually evaluated, palpated and scored for WS based on scoring system of Kuttappan et al. (2012) and WB based on the scoring system of Tijare et al. (2016).

Statistical Analysis

All data were subjected to an Analysis of Variance (ANOVA) using the General Linear Model Procedure (SPSS V24); pen was used at the experimental unit. Percentage and categorical data (mortality, processing yields, and WS and WB incidence data) were arc-sine transformed for analysis. Means that were significantly different at $p<0.05$ were separated using Duncan's Multiple Range Test.

Results

Performance

During the starter phase, supplementation of arginine at 136 dArg:dLys and the inclusion of 100 ppm Vitamin C significantly increased ($p < 0.001$) BW (Table 15) when compared to the control fed broilers. Broilers fed diets with Vitamin C at both inclusion levels had an elevated rate of consumption ($p = 0.008$) (Table 16) during the starter phase as compared to the control diet. This resulted in increased consumption of dig lysine ($p = 0.004$) during this same time period.

Table 15. Body weight and mortality of male broilers fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

	d 0	d 15	d 29	d 43	d 50	d 1-50
Treatment	g/bird	g/bird	------------- kg/bird		-----------------	%
Control	39.82	480.52[b]	1.590[a]	3.000[bc]	3.803	3.8
Reduced AA[1]	39.99	-----	1.542[b]	2.998[bc]	3.811	3.1
124 dArg:dLys[2]	39.92	480.99[b]	1.536[b]	2.995[bc]	3.841	5.0
136 dArg:dLys[3]	39.97	501.54[a]	1.547[ab]	3.033[abc]	3.838	4.7
100 ppm Vitamin C[4]	39.82	501.18[a]	1.590[a]	3.046[ab]	3.842	4.4
200 ppm vitamin C[5]	39.86	493.26[ab]	1.558[ab]	3.057[a]	3.864	3.4
Combination[6]	39.90	493.25[ab]	1.563[ab]	2.991[c]	3.801	2.2
PSEM	0.07	2.08	0.005	0.008	0.013	0.5
P-value	*0.561*	*<0.001*	*<0.001*	*0.022*	*0.547*	*0.743*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

During the grower phase (16 to 29 d-of-age), reducing the AA density by 15% reduced average BW ($p = 0.005$) compared to the control fed broilers. Supplementing the reduced density diet with the highest level of arginine and both levels of Vitamin C resulted in similar BW to the control. The reduction in AA density resulted in an increased in feed consumption ($p < 0.001$) during the grower phase of production as compared to the control. All treatments that included supplemental arginine or Vitamin C in the reduced AA grower diet also had elevated levels of consumption as compared to the control diet. Even with the elevated levels of consumption, all treatment fed broilers had a consumption rate of dig lysine (Table 18) significantly less ($p < 0.001$) than the control diet due to the 15% reduction in dig lysine. The increase in feed consumption in all treatment fed broilers resulted in an increased in mortality adjusted FCR ($p < 0.001$) (Table 17) during the grower phase of production as compared to the control diet. Cumulatively through 29 d of age, all broilers fed the reduced AA density diet with or without supplementation of arginine and Vitamin C had elevated feed consumption ($p < 0.001$), FCR($p < 0.001$), and decreased dig lysine intake ($p < 0.001$) compared to the control diet. Increasing the level of arginine to 136% in the amino acid reduced grower phase improved FCR ($p < 0.001$) through 29 d of age as compared to the reduced AA control diet and that of 124% arginine treatment.

Following the finisher phase (30 to 43 d-of-age), the addition of Vitamin C at the high level increased ($p = 0.022$) BW as compared to the control fed broilers while all other treatments were similar to the control. The rate of feed consumption during the finisher phase of production was similar for all dietary treatments as compared to the control with the exception of the combination of the low level of supplemental arginine and vitamin C which resulted in these broilers having the lowest average BW ($p = 0.022$) on d 43. This reduction in consumption was responsible for the reduction in dig lysine intake in this treatment as compared to the control diet. Improvements in FCR were observed during the finisher phase ($p < 0.001$) in all dietary treatments as compared to the control diet. Cumulatively through 43 d of age, all dietary treatments had similar rates of consumption ($p = 0.109$) and FCR compared to the control diet ($p = 0.110$). This indicates that compensatory gain following the reduction in AA density in the grower phase was sufficient to eliminate the growth reduction observed due to AA density. However, this reduction in AA density during the grower phase, did result in reductions in dig lysine intake through 43 d of age ($p < 0.001$).

Table 16. Feed consumption of male broilers fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

Treatment	Starter	Grower	Finisher	WD	Day 1-29	Day 1-43	Day 1-50
	----------	----------	----------	kg/bird	----------	----------	----------
Control	0.510^{bc}	1.578^{b}	2.502^{a}	1.535	2.082^{c}	4.585	6.120
Reduced AA[1]	---	1.662^{a}	2.456^{abc}	1.537	2.178^{ab}	4.635	6.172
124 dArg:dLys[2]	0.505^{c}	1.653^{a}	2.431^{abc}	1.530	2.158^{b}	4.590	6.120
136 dArg:dLys[3]	0.518^{ab}	1.664^{a}	2.448^{abc}	1.513	2.182^{ab}	4.630	6.143
100 ppm Vitamin C[4]	0.523^{a}	1.686^{a}	2.477^{ab}	1.517	2.209^{a}	4.686	6.203
200 ppm Vitamin C[5]	0.523^{a}	1.670^{a}	2.447^{abc}	1.522	2.193^{ab}	4.640	6.161
Combination[6]	0.518^{ab}	1.682^{a}	2.413^{c}	1.520	2.200^{ab}	4.613	6.133
PSEM	1.9	7.2	9.9	6.8	8.5	15.2	19.8
P-value	*0.005*	*0.001*	*0.040*	*0.848*	*<0.001*	*0.275*	*0.679*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

At the end of the withdraw phase (43 to 50 d-of-age) and cumulatively throughout the growout (1 to 50 days-of-age), all dietary treatments had similar BW (p = 0.220) and FC (p = 0.848 and p = 0.415, respectively) as compared to the control diet. During the withdrawal phase of production, no difference in dLys intake (p = 0.851) or FCR (p=0.067). Feed conversion ratio and dLys intake was impacted by dietary treatment. Increasing the digArg ratio to 124% decreased FCR when compared to the control fed broilers. The reduction in AA density by 15% during the grower phase did not negatively impact FCR as all dietary treatment had similar or lower FCR compared to the control diet. However, the reduction did reduce the digLys intake ($p<0.001$) in all of the dietary treatments cumulatively from 1 to 50 day of age. Even though the birds consumed less digLys, their performance was at least equivalent to the control fed broilers.

No differences were observed in mortality during any phase or cumulatively for the entire growout period.

Table 17. Feed conversion of male broilers fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

Treatment	Starter	Grower	Finisher	WD	Day 1-29	Day 1-43	Total d 1-50
Control	1.245	1.409^{c}	1.779^{a}	1.970	1.381^{d}	1.569	1.651^{ab}
Reduced AA[2]	---	1.577^{ab}	1.693^{bc}	1.938	1.472^{ab}	1.580	1.659^{a}
124 dArg:dLys[3]	1.238	1.588^{a}	1.671^{bc}	1.868	1.483^{a}	1.575	1.637^{abc}
136 dArg:dLys[4]	1.213	1.554^{b}	1.681^{bc}	1.886	1.451^{c}	1.562	1.625^{c}
100 ppm Vitamin C[5]	1.222	1.561^{ab}	1.706^{b}	1.953	1.459^{bc}	1.577	1.654^{ab}
200 ppm Vitamin C[6]	1.237	1.568^{ab}	1.657^{c}	1.928	1.469^{abc}	1.561	1.636^{bc}
Combination[7]	1.233	1.575^{ab}	1.702^{b}	1.916	1.469^{abc}	1.580	1.650^{ab}
PSEM	0.004	0.007	0.006	0.014	0.004	0.002	0.003
P-value	*0.083*	*<0.001*	*<0.001*	*0.067*	*<0.001*	*0.110*	*0.019*

a-d Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] Weight corrected FCR is corrected so that 1 point of FCR is equal to 32g of body weight.

[2] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[3] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[5] The level of dietary vitamin C was 94.38 ppm.

[6] The level of dietary vitamin C was 188.8 ppm.

[7] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

Table 18. Digestible lysine consumption of male broilers fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

Treatment	Starter	Grower	Finisher	WD	Day 1-29	Day 1-43	Day 1-50
	-------------------	-------------------	-------------------	g/bird	-------------------	-------------------	-------------------
Control	6.12^{bc}	17.20^{a}	24.53^{a}	13.81	23.25^{a}	47.78^{a}	61.59^{a}
Reduced AA[1]	---	15.46^{b}	24.07^{abc}	13.83	21.65^{bc}	45.72^{bc}	59.56^{b}
124 dArg:dLys[2]	6.06^{c}	15.38^{b}	23.83^{bc}	13.77	21.43^{c}	45.26^{c}	59.04^{b}
136 dArg:dLys[3]	6.22^{ab}	15.48^{b}	23.99^{abc}	13.61	21.69^{bc}	45.68^{bc}	59.30^{b}
100 ppm Vitamin C[4]	6.28^{a}	15.68^{b}	24.27^{ab}	13.66	21.96^{b}	46.23^{b}	59.88^{b}
200 ppm Vitamin C[5]	6.28^{a}	15.53^{b}	23.98^{abc}	13.69	21.80^{ab}	45.78^{bc}	59.48^{b}
Combination[6]	6.22^{ab}	15.65^{b}	23.65^{c}	13.68	21.86^{ab}	45.51^{bc}	59.19^{b}
PSEM	0.02	0.09	0.09	0.06	0.09	0.17	0.21
P-value	*0.004*	*<0.001*	*0.040*	*0.851*	*<0.001*	*<0.001*	*<0.001*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

Processing

Since average BW was similar at the conclusion of the trial, no differences were observed with regard to weights of the live, fasted broiler or carcass between any of the treatments (Table 19). Inclusion of supplemental arginine to increase the ratio of dArg to dLys to 136% increased leg weight as compared to the control diet. Similarly, the addition of arginine as well as the lower level of arginine and the combination treatment of arginine and Vitamin C increased leg yield compared to the control diet. The reduction in AA density during the grower phase did not significantly reduce breast weight or yield compared to the control diet. However, supplementing digArg to 124% along with the combination of 124% digLys with Vitamin C reduced breast meat yield percentage compared to the control diet. Therefore, the reduced consumption of digLys did not impact breast meat yield significantly in the remaining dietary treatments.

Table 19. Processing weights and processing yields of male broilers fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

	Processing Weights						Processing Yields, %				
Treatment	Live, Fasted, kg	Chilled carcass, kg	Breast, g	Tender, g	Leg, g	Wing, g	Chilled Carcass	Breast	Tender	Leg	Wing
Control	3.705	2.942	950.40	168.70	0.85^{b}	0.30	79.40	32.29^{a}	5.75	28.99^{b}	10.04
Reduced AA[1]	3.716	2.945	936.50	169.40	0.86^{b}	0.30	79.26	31.78^{abc}	5.76	29.14^{b}	10.04
124 dArg:dLys[2]	3.681	2.922	920.30	167.90	0.87^{b}	0.30	79.38	31.46^{c}	5.75	29.63^{a}	10.18
136 dArg:dLys[3]	3.764	3.000	952.90	166.70	0.89^{a}	0.30	79.82	31.69^{abc}	5.56	29.62^{a}	10.08
100 ppm Vitamin C[4]	3.739	2.961	950.20	170.10	0.87^{ab}	0.30	79.17	32.09^{ab}	5.75	29.35^{ab}	10.07
200 ppm Vitamin C[5]	3.740	2.961	949.60	169.20	0.87^{ab}	0.29	79.17	32.05^{abc}	5.72	29.35^{ab}	10.03
Combination[6]	3.716	2.959	931.70	168.20	0.87^{ab}	0.30	79.61	31.49^{bc}	5.69	29.55^{a}	10.05
PSEM	12.5	9.710	4.43	0.84	3.08	1.00	0.09	0.08	0.03	0.06	0.02
P-value	*0.550*	*0.393*	*0.232*	*0.924*	*0.029*	*0.502*	*0.312*	*0.021*	*0.157*	*0.006*	*0.614*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C

White Striping and Woody Breast

Compared to the control-fed birds (Trt 1), the average woody breast score was significantly reduced ($p < 0.006$) by the supplementation of arginine at a level of 124 dArg:dLys individually, and in combination with 100 pm vitamin C (Table 20). Reviewing incidence of WB scores for each treatment, it is apparent that there was a significant downward shift (i.e. an improvement) in the incidence of filets showing sever signs of WB (scores of 3 ($p = 0.010$)) (Table 21). Reducing the AA density in the grower phase of production alone or in combination with an increased dArg to dLys ratio significantly reduced the percentage of filets showing severe WB characteristics.

A similar response to dietary treatments were observed when evaluating the presence and severity of WS in breast filets. Birds fed a reduction in amino acid density during the grower phase and the inclusion of 124 dArg:dLys significantly reduced the average white striping score (1.20) compared to the control fed broilers (1.38) (Table 22). The dietary treatment of 124% dArg to dLys was the only treatment to have filets that were absent of WS. The inclusion of 124% dArg:dLys alone or combined with Vitamin C significantly increased the percentage of filets that exhibit normal or slight WS (scores of 0 and 1) as compared to control fed broilers.

Table 20. Meat quality measurements and woody breast profile of male broilers that are fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

	Meat Quality Measurements	
Treatment	Ave. Woody Breast Score	Ave. White Striping Score
Control	1.70[a]	1.38[ab]
Reduced AA[1]	1.66[a]	1.35[abc]
124 dArg:dLys[2]	1.37[b]	1.20[c]
136 dArg:dLys[3]	1.48[ab]	1.32[abc]
100 ppm Vitamin C[4]	1.65[a]	1.39[ab]
200 ppm Vitamin C[5]	1.68[a]	1.46[a]
Combination[6]	1.39[b]	1.25[bc]
PSEM	0.03	0.02
P-value	*0.006*	*0.011*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

Table 21. Woody breast profile of male broilers that are fed diets with a higher ratio of dArg: dLys, vitamin C and/or a lower digestible amino acid ratio

	Woody Breast Profile							
Treatment	Score of 0, %	Score of 0.5, %	Score of 1, %	Score of 1.5, %	Score of 2, %	Score of 3, %	Score of 0&1, %	Score of 2&3, %
Control	1.10	1.10	43.30	0.00	37.50	16.50[a]	45.50[ab]	54.00
Reduced AA[1]	0.00	0.00	39.80	2.20	52.30	5.50[bc]	42.00[b]	57.80
124 dArg:dLys[2]	3.50	3.30	55.20	1.10	32.30	3.89[bc]	63.10[a]	37.33
136 dArg:dLys[3]	2.20	0.00	51.90	3.30	37.00	6.11[bc]	57.40[ab]	47.22
100 ppm Vitamin C[4]	0.00	2.20	41.00	1.10	45.50	9.90[abc]	44.30[b]	55.40
200 ppm Vitamin C[5]	1.10	1.10	39.90	0.00	45.30	12.10[ab]	42.10[b]	57.40
Combination[6]	0.00	4.40	54.60	0.00	38.80	2.20[c]	59.00[ab]	41.00
PSEM	0.004	0.006	0.02	0.003	0.02	0.01	0.02	0.02
P-value	*0.162*	*0.307*	*0.142*	*0.528*	*0.164*	*0.010*	*0.044*	*0.078*

a-c Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

Table 22. White striping profile of male broilers that are fed diets with a higher ratio of dArg: dLys, vitamin C and/ or a lower digestible amino acid ratio

	White Striping Profile							
Treatment	Score of 0, %	Score of 0.5, %	Score of 1, %	Score of 1.5, %	Score of 2, %	Score of 2.5, %	Score of 0&1, %	Score of 2&2.5, %
Control	0.00[b]	0.00	38.86	46.66	12.21	2.22	38.86[b]	61.09
Reduced AA[1]	0.00[b]	1.11	45.55	41.10	7.77	4.44	46.66[ab]	53.31
124 dArg:dLys[2]	4.58[a]	2.22	52.78	30.39	9.99	0.00	59.58[a]	40.38
136 dArg:dLys[3]	0.00[b]	0.00	50.70	34.56	12.35	1.11	50.70[ab]	48.02
100 ppm Vitamin C[4]	0.00[b]	0.00	43.33	37.77	17.77	1.11	43.33[ab]	56.65
200 ppm Vitamin C[5]	0.00[b]	0.00	38.88	35.54	22.21	3.33	38.88[b]	61.08
Combination[6]	0.00[b]	0.00	56.67	36.65	4.44	2.22	57.78[a]	43.31
PSEM	0.004	0.003	0.02	0.02	0.02	0.005	0.02	0.02
P-value	*0.007*	*0.316*	*0.239*	*0.458*	*0.101*	*0.447*	*0.049*	*0.061*

a-b Means in columns with different groupings differ significantly at $p \leq 0.05$

[1] The digestible amino acid content of the Grower diet (d 13-24) was reduced by 15% when compared to the Control diet (Treatment 1); however, the same Starter, Finisher and Withdraw diets from Treatment 1 were fed.

[2] The dig Arg:dig Lys ratio was increased from 112 to 136 in the starter, 111 to 136 in the grower, 112 to 136 in the finisher, and 110 to 136 in the withdraw phase.

[4] The level of dietary vitamin C was 94.38 ppm.

[5] The level of dietary vitamin C was 188.8 ppm.

[6] Birds were fed, the starter, finisher and withdraw from Treatments 3 and 5, and the grower from Treatment 2; however, all were supplemented with dietary arginine and Vitamin C.

Discussion

Woody breast and white striping have been well defined in recent publications by Kuttappan et al. (2013), Shivo et al. (2014), and Kuttappan et al. (2016). These authors are in agreement that WB and WS are expressed by rapidly-growing and high yielding genetic strains that are grown for a longer period of time to reach the heavier weight classes. Congruently, Kuttappan et al. (2017) noted that the incidence of WS, WB, and petechial hemorrhagic lesions; increases as the broiler becomes heavier and older. Although WB and WS have been reported to have similar macroscopic and microscopic similarities to other conditions such as, hereditary muscular dystrophy and nutritional myopathy, the etiologies do not appear to be congruent (Kuttappan et al., 2016). However, previous research has noted the similarities in lesions between WB and WS including; myodegeneration, necrosis, lipidosis, infiltration of marcophages and lymphocytes, and fibrosis. Unfortunately, the mechanism responsible for such lesions has yet to be unequivocally determined.

While these myopathies are not a food safety issue, they have become a consumer acceptability issue. A study conducted by Kuttappan et al. (2012a) noted that consumers described the appearance of WS as “fatty”, resulting in a negative impact upon the healthy image of breast fillets. Furthermore, 50% of consumers stated they would be less likely to purchase fillets with any degree of white striping. This rejection of breast fillets in fresh retail could result in a $200 million loss in profits for the poultry industry (Gee, 2016). Unfortunately, this loss has the potential to rise with the new proposed rule by FSIS in regards to inflamed woody breast and white striping. It states that any inflamed WB or severe WS, which includes; swollen breast tissues, scattered blood spots, or presence of gelatinous fluid, will be considered unadulterated. Any adulterated meat is considered unfit for human consumption and will either be trimmed or completely discarded (USDA, 2017).

This study sought to define inclusion levels of nutritional strategies found in Chapter 2 that might lead to the elimination or a significant reduction in one or both conditions. Nutrients were selected based on their ability to elicit nutritional and physiological responses and properties. However, in order to fully understand how these nutrients impact WS and WB, the cellular and muscle structure must be fully understood. Satellite cells are the basis of muscle development and have the capability to repair damaged muscle fibers. Satellite cells are located underneath the membrane of the muscle fiber and are considered to be myogenic precursors of postnatal and adult skeletal muscle (Mauro, 1979; Campion, 1984). During the embryonic stage these cells proliferate, adding nuclei to the fibers (Moss and LeBlond, 1971). However, as the animal matures, satellite cells become mitotically quiescent and only begin to divide in response to injury (Mauro, 1979; Campion, 1984). During these times of injury, some satellite cells are lost while the remainder survives by migrating to regions of the muscle where conditions are ideal. This migration is crucial in the satellite cells regeneration process during muscle fiber injury.

In this study, L-Arginine was supplemented at two different levels based off a reference level established in the previous experiment. The inclusion of arginine at

1.25% into a broiler diet has shown to improve live weight, feed conversion, and carcass yield (Corzo et al., 2003). Congruently, Fernandez et al. (2009) reported that arginine supplemented at 1.30 dArg:dLys, exceeding the NRC recommendation of 1.05 dArg: dLys, improved muscle development in broilers but had no effect on the carcass yield of broilers on d 42. This data suggests that dietary supplementation of arginine may be beneficial in maximizing breast yield in broiler chickens. Furthermore, arginine is a substrate for nitric oxide production, a powerful vasodilator. Bautista-Ortega and Ruiz-Feria (2010) demonstrated that feeding higher levels of arginine causes vasodilation via an increase in nitric oxide (NO) production. Jobgen et al. (2006) theorized that as a result of this vasodilation, the efficiency of nutrient and metabolite exchange in the poorly vascularized breast tissue could be improved.

The production of nitric oxide has the capability to improve metabolic disorders within the broiler; this includes pulmonary hypertension syndrome (PHS) or better known as ascites. This disorder develops in broilers when elevated pulmonary vascular resistance forces the right ventricle to increase pulmonary arterial pressure (PAP). Pulmonary hypertension syndrome is a metabolic disorder that commonly effects fast growing broilers and is often found in hypoxic or hypoxemic conditions (Khajali and Wideman, 2010). Arginine has been observed to impact PHS by markedly increasing the NO production and reducing the PAP in broilers (Tan et al., 2005). Similarly, Bautista-Ortega and Ruiz-Feria (2010) observed a reduction in pulmonary artery reactivity in broilers fed a diet containing arginine, vitamin C and vitamin E. These data suggest that arginine improved flow of oxygen in the blood which in turn positively impacted the hypoxic state of the muscle.

Recent research has reported that WB can be associated with localized hypoxia due to a reduction in capillary supply (Bilgili, 2013; Mutryn et al., 2015). Numerous genes have been found to be regulated by Hypoxia-inducible factor-1 gene (HIF-1) in broilers with WB, suggesting a hypoxic state within the muscle (Mutryn et al., 2015). Hypoxia-inducible factor-1 gene is an important transcription factor due to its ability to regulate cell proliferation and critical in maintaining oxygen homeostasis within the cell (Shaw, 2008; Brahimi-Horn et al., 2011). Among the genes that are up-regulated by HIF-1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, PLOD2, has been observed to have negative consequences upon the cellular structure of birds affected with WB. PLOD2 is associated with the extracellular matrix composition, more specifically the stiffening and collagen alignment components (Mutryn et al., 2015). This upregulation of PLOD2, increases collagen content which in turn enhances cell adhesion and elongation, creating a stiff cellular environment (Gilkes et al., 2013). This histological change is congruent with previous reports of lesions such as fibrosis that is present in WB affected birds.

Lastly, hypoxic conditions have a tendency to stimulate satellite cell proliferation (Urbani et al., 2012), which as mentioned previously, is critical for skeletal muscle regeneration. The stimulation of the cells under hypoxic conditions has the ability to result in muscle hypertrophy during myogenesis and myoregeneration, which has been observed microscopically by Mutryn et al. (2015). The previously mentioned studies suggest that when hypoxic conditions are present in the muscle, the inclusion of arginine reduces hypoxia; in turn reducing WB severity. In this study, the supplementation of dArg: dLys at 136 significantly improved performance in the early stages of growth compared to 124 dArg:dLys. However, this trend did not continue in regards to meat quality; the inclusion at 124% significantly reduced the severity of WB and WS compared to the control, while broilers fed 136% level produced similar results to that of the control. These results suggest that while arginine did increase muscle development and breast yield (Fernandez et al., 2009) at 136%; it also led to a surge in NO production potentially overwhelming the immune system (Khajali and Wideman, 2010). This surge could have led to the inability to reduce hypoxic conditions effectively; in turn resulting in higher severity of WB.

Furthermore, Vignale et al. (2017) reported that birds with severe WS had lower expression of insulin growth factor – 1 (IGF-1) when compared to normal breast fillets. Insulin growth factor – 1 is widely studied due to its ability to activate muscle hypertrophy and metabolism of skeletal muscles such as satellite cells. Sacheck et al. (2004) noted that IGF-1 down regulates MuRF1 and antrogin-1 genes which are responsible for protein degradation within the muscle. In the current study, a reduction in the severity of WS was observed in broilers fed a diet of 124 dArg:dLys. Arginine plays an important role as a protein constituent that is involved in secretion of growth hormone, specifically IGF-1 (Fernandes et al., 2009). It can be suggested that the supplementation of arginine at a lower level combatted the suppression of IGF-1 as seen in severe WS and in turn reduced the severity of WS.

Additionally, the supplementation of vitamin C has been recommended to reduce the incidence and severity of WB (Abasht et al., 2016). Vitamin C, or ascorbic acid, is a well-known anti-oxidant that serves in a multitude of enzymatic reactions and is essential in the biosynthesis of collagen (Ishikawa et al., 2013). Furthermore, vitamin C has the ability to protect cells from free radicals that are derived from reactive oxygen species (ROS). These molecules can cause detrimental damage to DNA, proteins, lipids, and carbohydrates (Surai, 2002). In a normal environment, broilers are able to sufficiently synthesize vitamin C to meet its physiological needs; however, during stress it is unable to maintain optimal balance. Recently, Abasht et al. (2016) hypothesized that glycogen depletion that is commonly seen in WB affected birds can be contributed to the over stimulation of the ascorbic acid biosynthesis pathway. Abasht et al. (2016) concluded the report with a recommendation to supplement vitamin C in order to decrease the activity of the pathway and reduce the damage occurring within the breast tissue.

Oxidative stress is regarded as the cause of several pathologies that affect poultry growth. Mutryn et al. (2015) suggested that oxidative stress could be a major contributor to WB. This occurs through an increase in ROS after major muscle disuse or mitochondrial dysfunction (Powers et al., 2010; Balaban et al., 2005). The increase in ROS, as mentioned previously, is detrimental to muscle cells due to cytotoxicity and can damage or alter proteins and membrane lipids (Powers et al., 2010). Furthermore, the increase in ROS can impair cellular signaling pathways that are critical in acting upon skeletal muscle remodeling (Powers et al., 2010). It can be hypothesized that the inclusion of vitamin C into the diet may aid in the protection of cells from free radicals and oxidative damage to the muscle tissue.

Additionally, oxidative stress is involved in the etiology of PHS (Peacock et al., 1990; Maxwell et al., 1992; Bottje and Wideman, 1995). The reaction of superoxide anion with nitric oxide results in the production of a potent oxidant agent that is responsible for direct tissue damage (Beckman et al., 1990; Szabo, 1996). A study conducted by Ruiz-Feria (2009) reported that supplementation of arginine, vitamin E, and vitamin C significantly improved pulmonary vasodilation and increased blood flow in broilers. This data suggests these ingredients worked in synergy by increasing the NO bioavailability with the inclusion of arginine providing extra substrate for NO production, and vitamin E and vitamin C providing protection against oxidative stress. Similarly, in the current trial, birds fed a reduced amino acid density diet with the inclusion of 124 dArg:dLys and 100 ppm vitamin C, were observed to significantly reduce the severity of WB when compared to the control diet.

Furthermore, the reduction in amino acid density in the grower phase was believed to maximize satellite cell development to allow the breast tissue to recover from rapid growth in the starter phase (Powell et al., 2014). Amino acids role within a broiler diet has been well established. Regulation of key metabolic pathways, optimization of muscle growth, and enhancement of protein synthesis are just a few roles amino acids possess within muscle development of a broiler (Wu, 2009). Previous studies have reported that the alteration of amino acids within a diet has shown to impact satellite cell activity (Pophal et al., 2004; Powell et al., 2013). Decreasing the amino acid density in the current study reduced BW and FCR throughout the grower phase; however compensatory gain resulted in similar growth performance parameters to the control at the conclusion of the experiment. These results are congruent with previous studies which found when fed a reduced amino acid density diet; broilers experience a decrease in BW, FCR, and carcass yield (Kidd et al., 2005). Data from the previous experiment (Chapter 2) suggested that lowering the amino acid density during the grower phase, allows the muscle to recover from rapid growth experienced during the starter phase by reducing the satellite cell activity. During the current study, reducing the amino acid density during the grower phase did not impact the final growth performance. However, when fed in conjunction with 124 dArg:dLys and 100 ppm vitamin C supplementation, a reduction in the severity of WB was observed without negatively impacting breast filet weight.

These data indicate that the nutritional strategies including an increase in digestible arginine, inclusion of vitamin C, and a reduction in AA density within the diet could be possible strategies to mitigate WB and WS. The hypothesized mechanism of each strategy is distinct and thus combining strategies may be the most effective. However, it is unclear and unlikely that nutritional strategies will completely eliminate the incidence of WB and WS however; mitigation through nutrition can be a valuable tool in rearing high yielding broilers.

CHAPTER IV

CONCLUSION

Woody breast and white striping have become a growing issue in recent years due to the negative impact upon consumer acceptability and revenue. However, a reduction in growth rate and breast yield is not feasible with the growing consumer demand. Other solutions such as improvements in genetic selection, nutrition, and management tools are all potential possibilities to mitigate these muscle myopathies.

The inclusion of arginine, vitamin C, and the reduction of the amino acid density during the grower phase within a broiler diet has shown to positively impact the incidence of woody breast and white striping compared to an industry standard diet. These nutritional strategies improved the *Pectoralis major* by reducing the amount of severe WS and WB scores.

Arginine included at 124% during the first experiment significantly reduced woody breast without negatively impacting growth performance. Similarly, arginine included at 124% during the second experiment significantly reduced woody breast and white striping severity. This treatment in the second experiment reduced breast meat yield although no differences were observed in filet weight. The reduction in woody breast may be attributed to arginine's ability to increase the blood flow within the breast tissue by vasodilation of the blood capillaries. This increase in blood flow allows more oxygen to be carried through the blood stream and reduce the hypoxic state within the muscle.

The inclusion of 100 ppm vitamin C within the broiler diet significantly reduced the severity of woody breast during the first experiment while not impacting growth performance. Vitamin C eliminates free radicals that cause damage to the DNA, carbohydrates, proteins, and membrane lipids. By reducing these free radicals, the oxidative stress condition within the breast tissue may be significantly decreased. During the second experiment the inclusion of vitamin C at 100 ppm and 200 ppm did not influence the degree of WB or WS compared to the control diet. However, the reduction in protein during the grower phase in experiment 2 may have influenced this response. The influence of vitamin C on woody breast may be attributed to vitamin C's ability to counteract the oxidative stress occurring within the *Pectoralis major*.

Lastly, the reduction in amino acid density by 15% during the grower phase in Experiment I significantly reduced the severity of woody breast. By reducing the amino acid density it allows the satellite cells to recover and repair the muscle fibers after the rapid growth occurred during the starter phase.

Further studies will need to be conducted on the severity and incidence of hypoxia and oxidative stress within woody breast to determine the effectiveness of vitamin C and arginine. Additionally, the study of satellite cells in the breast tissue throughout the growth phases of the broiler will need to be conducted to evaluate the muscle fiber damage and repair rate present in woody breast.

In conclusion, the supplementation of arginine, vitamin C, and/ or the reduction of amino acid density during the grower phase has shown to reduce the severity of woody breast and white striping although some of these strategies were observed to reduced yield percentage but not filet weight. While the mechanism responsible remains unknown, the reduction in woody breast and white striping severity has the potential to lower the tremendous revenue losses, increase the amount of saleable meat, and improve consumer acceptance with the improvement in the severity of these muscle myopathies.

REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PLoS One 11:e0153750.

Abdukalykova, S. T., X. Zhao, and C. A. Ruiz-Feria. 2008. Arginine and vitamin modulate the subpopulations of T lymphocytes in broiler chickens. Poult. Sci. 87: 50-55.

Agudelo, L. 1983. Possible causes of avian oedema. Int. J. Poult. Sci. 22:8-14.

al-Taweil, R. N., and A. Kassab. 1990. Effect of dietary vitamin C on ascites in broiler chicks. Int. J Vitam. Nutr. Res 60:366-371.

Allen, D. G., G. D. Lamb, and H. Westerblad. 2008. Impaired calcium release during fatigue. J. Appl. Physiol. 104:296-305.

Ali, M. S., G. Kang, and S. T. Joo. 2008. A review: Influences of pre-slaughter stress on poultry meat quality. Asian-Australas. J. Anim. Sci. 21:912.

Austic, R., and C. Calvert. 1981. Nutritional interrelationships of electrolytes and amino acids. Fed. Proc. 40:63-67.

Avanzo, J. L., C. X. de Mendonça Jr, S. M. P. Pugine, and M. de Cerqueira Cesar. 2001. Effect of vitamin E and selenium on resistance to oxidative stress in chicken superficial pectoralis muscle. Comp. Biochem. Physiol. C Toxicol. & Pharmacol. 129:163-173.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching1. Poult. Sci. 73:1441-1447.

Balaban, R. S., S. Nemoto, and T. Finkel. 2005. Mitochondria, oxidants, and aging. Cell 120:483-495.

Ball, R. O., K. L. Urschel, and P. B. Pencharz. 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. The Journal of Nutrition 137:1626S-1641S.

Barbut, S. 2015. The Science of Poultry Meat and Processing. University of Guelph. Chapter 15. ISBN 978-0-88955-626-3.

Bashir, I. N., M. A. Munir, M. A. Saeed, A. Raza, and F. K. Raza. 1998. Immunomodulatory effects of water soluble vitamins on heat stressed broiler chickens. Indian Journal of Animal Nutrition 15:11-17.

Bauermeister, L. J., A.U. Morey, E. T. Moran, M. Singh, C. M. Owens, S. R. McKee. 2009. Occurrence of WS in chicken breast fillets in relation to broiler size. Poult. Sci. 88 104 (Abstr.)

Bautista-Ortega, J., and C. A. Ruiz-Feria. 2010. l-Arginine and antioxidant vitamins E and C improve the cardiovascular performance of broiler chickens grown under chronic hypobaric hypoxia. Poult. Sci. 89:2141-2146.

Beckman, J. S., T. W. Beckman, J. Chen, P. A. Marshall, and B. A. Freeman. 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. 87:1620-1624.

Bedford, M. R., and J. D. Summers. 1985. Influence of the ratio of essential to non-essential amino acids on performance and carcass composition of the broiler chick. Br. Poult. Sci. 26:483-491.

Bendich, A., L. Machlin, O. Scandurra, G. Burton, and D. Wayner. 1986. The antioxidant role of vitamin C. Free Radic. Biol. Med. 2:419-444.

Bilgili, S. 2013. Broiler chicken myopathies: II. Woody breast. Worthw Oper Guidel Suggest 1.

Boorman, K. 1971. The renal reabsorption of arginine, lysine and ornithine in the young cockerel (Gallusdomesticus). Comp. Biochem. Physiol., Part A Physiol. 39:29-38.

Bottje, W. G., and R. F. Wideman Jr. 1995. Potential role of free radicals in the pathogenesis of pulmonary hypertension syndrome. Avian Biol Res (United Kingdom).

Bowker, B., and H. Zhuang. 2017. Woody breast condition in broiler breast meat. USDA-ARS, U.S. National Poultry Research Center.

Bowker, B., and H. Zhuang. 2016. Impact of white striping on functionality attributes of broiler breast meat1. Poult, Sci. 95:1957-1965.

Brahimi-Horn, M. C., G. Bellot, and J. Pouysségur. 2011. Hypoxia and energetic tumor metabolism. Curr. Opin. Genet. Dev. 21:67-72.

Brewer, V. B., V. A. Kuttappan, J. L. Emmert, J. F. C. Meullenet, and C. M. Owens. 2012. Big-bird programs: Effect of strain, sex, and debone time on meat quality of broilers. Poult. Sci. 91:248-254.

Campion, D. R. 1984. The Muscle Satellite Cell: A Review. Pages 225-251 in International Review of Cytology. G. H. Bourne, and J. F. Danielli eds. Academic Press.

Chatterjee, D., H. Zhuang, B. C. Bowker, A. M. Rincon, and G. Sanchez-Brambila. 2016. Instrumental texture characteristics of broiler pectoralis major with the wooden breast condition1. Poult. Sci. 95:2449-2454.

Corzo, A., C. Fritts, M. Kidd, and B. Kerr. 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Anim. Feed Sci. Technol. 118:319-327.

Corzo, A., J. E. T. Moran, and D. Hoehler. 2003. Arginine need of heavy broiler males: applying the ideal protein concept. Poult. Sci. 82:402-407.

Corzo, A., J. E. T. Moran, and D. Hoehler. 2002. Lysine need of heavy broiler males applying the ideal protein concept. Poult. Sci. 81:1863-1868.

Crews J. 2016. Sanderson addresses woody breast challenges. Available at: http://www.meatpoultry.com/articles/news_home/Business/2016/08/Sanderson_addresses_woody_brea.aspx?ID=%7B215346A4-7ADF-4548-A6BD-B8EF62E3036C%7D. Accessed July. 2017.

Davis, T. A., and M. L. Fiorotto. 2009. Regulation of muscle growth in neonates. Curr Opin Clin Nutr Metab Care 12:78.

de Brot S. Perez S. Shivaprasad H. L. Baiker K. Polledo L. Clark M. Grau-Roma L. 2016 Wooden breast lesions in broiler chickens in the UK Vet Rec. 1 pii: vetrec-2015-103561.

Dietert, R. R., K. A. Golemboski, and R. E. Austic. 1994. Environment-immune interactions. Poult. Sci. 73:1062-1076.

Dransfield, E., and A. A. Sosnicki. 1999. Relationship between muscle growth and poultry meat quality. Poult. Sci. 78:743-746.

Dröge, W. 2002. Aging-related changes in the thiol/disulfide redox state: implications for the use of thiol antioxidants. Exp. Gerontol 37:1333-1345.

Duclos M. J., C. Berri. E.Le Bihan-Duval. 2007. Muscle growth and meat quality. J. Appl. Poult. Res. 16:107–112.

Dudzinski, D. M., and T. Michel. 2007. Life history of eNOS: partners and pathways. Cardiovasc. Res. 75:247-260.

Elstner, E. 1991. Oxygen radicals—biochemical basis for their efficacy. Journal of Molecular Medicine 69:949-956.

Emadi, M., F. Jahanshiri, K. Kaveh, M. Hair-Bejo, A. Ideris, and A. R. Alimon. 2011. Nutrition and immunity: the effects of the combination of arginine and tryptophan on growth performance, serum parameters and immune response in broiler chickens challenged with infectious bursal disease vaccine. Avian Pathol. 40:63-72.

Fellenberg, M.A., H. Speisky. 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. Worlds Poult Sci J, 62: 53–70.

Fernandes, J. I. M., A. E. Murakami, E. N. Martins, M. I. Sakamoto, and E. R. M. Garcia. 2009. Effect of arginine on the development of the pectoralis muscle and the diameter and the protein:deoxyribonucleic acid rate of its skeletal myofibers in broilers. Poult. Sci. 88:1399-1406.

Ferreira, T. Z., R. A. Casagrande, S. L. Vieira, D. Driemeier, and L. Kindlein. 2014. An investigation of a reported case of white striping in broilers. J Appl Poult Res 23:748-753.

Florou-Paneri, P., I. Nikolakakis, I. Giannenas, A. Koidis, E. Botsoglou, V. Dotas, and I. Mitsopoulos. 2005. Hen performance and egg quality as affected by dietary oregano essential oil and tocopheryl acetate supplementation. Int. J. Poult. Sci. 4:449-454.

Fouad, A., H. El-Senousey, X. Yang, and J. Yao. 2013. Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. Animal 7:1239-1245.

Franchini, A., F. Sirri, N. Tallarico, G. Minelli, N. Iaffaldano, and A. Meluzzi. 2002. Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamins E and C. Poult. Sci. 81:1744-1750.

Gee, K. 2016. Poultry's tough new problem:'Woody Breast'. Wall Street Journal. Sect. Business and Tech 267:B1.

Gerrard, D. E., and A. L. Grant. 2003. Principles of animal growth and development. Kendall Hunt.

Gilkes, D. M., S. Bajpai, P. Chaturvedi, D. Wirtz, and G. L. Semenza. 2013. Hypoxia-inducible factor 1 (HIF-1) promotes extracellular matrix remodeling under hypoxic conditions by inducing P4HA1, P4HA2, and PLOD2 expression in fibroblasts. J. Biol. Chem. 288:10819-10829.

Goll, D. E., V. F. Thompson, R. G. Taylor, and J. A. Christiansen. 1992. Role of the calpain system in muscle growth. Biochimie 74:225-237.

Guney, M., B. Oral, H. Demirin, N. Karahan, T. Mungan, and N. Delibas. 2007. Protective effects of vitamins C and E against endometrial damage and oxidative stress in fluoride intoxication. Clin. Exp. Pharmacol. Physiol. 34:467-474.

Gutteridge, J. M., and B. Halliwell. 1989. 1 Iron toxicity and oxygen radicals. Baillieres Clin. Haematol. 2:195-256.

Halliwell, B. 1992. Reactive oxygen species and the central nervous system. J. Neurochem. 59:1609-1623.

Harper, A. E., N. J. Benevenga and R. M. Wohlhueter, 1970. Effects of ingestion of disproportional amounts of amino acids. Physiol. Rev. 50: 428-558.

Harthan, L. B., D. C. McFarland, and S. G. Velleman. 2013. The effect of nutritional status and myogenic satellite cell age on turkey satellite cell proliferation, differentiation, and expression of myogenic transcriptional regulatory factors and heparan sulfate proteoglycans syndecan-4 and glypican-1. Poult. Sci. 93:174–86.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003a. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500-1508.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003b. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509-1518.

Hickling, D., W. Guenter, and M. E. Jackson. 1990. The effects of dietary methionine and lysine on broiler chicken performance and breast meat yield. Can. J. Anim. Sci. 70:673-678.

Hidiroglou, N., N. Cave, A. Atwal, E. Farnworth, and L. McDowell. 1992. Comparative vitamin E requirements and metabolism in livestock. Proc. Annales de Recherches Veterinaires.

Hoving-Bolink, A. H., R. W. Kranen, R. E. Klont, C. L. M. Gerritsen, and K. H. de Greef. 2000. Fibre area and capillary supply in broiler breast muscle in relation to productivity and ascites. Meat Sci. 56:397-402.

Ishikawa, Y., and H. P. Bächinger. 2013. A molecular ensemble in the rER for procollagen maturation. Biochim. Biophys. Acta 1833:2479-2491.

Iqbal, M., U. Giri, D. K. Giri, M. S. Alam, and M. Athar. 1999. Age-dependent renal accumulation of 4-hydroxy-2- nonenal (HNE)-modified proteins following parenteral administration of ferric nitrilotriacetate commensurate with its differential toxicity: implications for the involvement of HNE–protein adducts in oxidative stress and carcinogenesis. Arch. Biochem. Biophys. 365:101-112.

Jobgen, W. S., S. K. Fried, W. J. Fu, C. J. Meininger, and G. Wu. 2006. Regulatory role for the arginine–nitric oxide pathway in metabolism of energy substrates. J. Nutr. Biochem. 17:571-588.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry – A review. Vet. J. 169:350-369.

Khajali, F., and R. F. Wideman. 2010. Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. Worlds Poult Sci J 66:751-766.

Kidd, M. T., A. Corzo, D. Hoehler, E. R. Miller, and R. W. A. Dozier. 2005. Broiler responsiveness (Ross x 708) to diets varying in amino acid density 123. Poult. Sci. 84:1389-1396.

Kidd, M. 2004. Nutritional modulation of immune function in broilers. Poult. Sci. 83:650-657.

Kuttappan, V. A., W. Bottje, R. Ramnathan, S. D. Hartson, C. N. Coon, B.-W. Kong, C. M. Owens, M. Vazquez-Añon, and B. M. Hargis. 2017. Proteomic analysis reveals changes in carbohydrate and protein metabolism associated with broiler breast myopathy. Poult. Sci. 96:2992-2999.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724-2733.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331-338.

Kuttappan, V. A., Y. S. Lee, G. F. Erf, J. F. C. Meullenet, S. R. McKee, and C. M. Owens. 2012a. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult. Sci. 91:1240-1247.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012b. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677-2685.

Kuttappan, V., V. Brewer, F. Clark, S. McKee, J. Meullenet, J. Emmert, and C. Owens. 2009. Effect of white striping on the histological and meat quality characteristics of broiler fillets. Poult. Sci 88:136-137.

Kwak, H., R. Austic, and R. Dietert. 1999. Influence of dietary arginine concentration on lymphoid organ growth in chickens. Poult. Sci. 78:1536-1541.

Larry, L. 2002. Industry Studies. 3 ed. M.E. Sharpe, Inc., Washington, DC.

Leclercq, B. 1998. Lysine: Specific effects of lysine on broiler production: comparison with threonine and valine. Poult. Sci. 77:118-123.

Leeson, S., J. D. Summers, and L. J. Caston. 1991. Diet dilution and compensatory growth in broilers. Poult. Sci. 70:867-873.

Lorenzi M. Mudalal S. Cavani C. Petracci M. 2014 Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy J. Appl. Poult. Res. 23 754758

Lykkesfeldt, J., and O. Svendsen. 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet. J. 173:502-511.

Macrae, V. E., M. Mahon, S. Gilpin, D. A. Sandercock, and M. A. Mitchell. 2006. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (Gallus domesticus). Br. Poult. Sci.47:264-272.

Mann, C. J., E. Perdiguero, Y. Kharraz, S. Aguilar, P. Pessina, A. L. Serrano, and P. Munoz-Canoves. 2011. Aberrant repair and fibrosis development in skeletal muscle. Skelet. Muscle 1:21.

Marks., J. 1975. A guide to the vitamins: their role in health and disease. Medical and Technical Publishing Co. Ltd, London, UK.

Mauro, A., 1979. Muscle regeneration. Raven Press.

Maxwell, M. H., Robertson, G. W., & McCorquodale, C. C. (1992). Whole blood and plasma viscosity values in normal and ascitic broiler chickens. Br. Poult. Sci. 4: 871-877.

Mazzoni, M., M. Petracci, A. Meluzzi, C. Cavani, P. Clavenzani, and F. Sirri. 2015. Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poult. Sci. 94:123-130.

McCall, M. R., and B. Frei. 1999. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radic. Biol. Med. 26:1034-1053.

McDowell, L. R. 1992. Minerals in animal and human nutrition. Academic Press Inc.

Mezes, M., P. Surai, G. Salyi, B. Speake, T. Gaal, and A. Maldjian. 1997. Nutritional metabolic diseases of poultry and disorders of the biological antioxidant defence system. Acta Vet. Hung 45:349-360.

Moran, J. E. T., and S. F. Bilgili. 1990. Processing losses, carcass quality, and meat yields of broiler chickens receiving diets marginally deficient to adequate in lysine prior to marketing1. Poult. Sci. 69:702-710.

Moss, F. P., and C. P. Leblond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421-435.

Mudalal, S., M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci. 2014. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal 9:728-734.

Mutryn, M. F., E. M. Brannick, W. Fu, W. R. Lee, and B. Abasht. 2015. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genomics 16:399.

National Chicken Council. 2016. About the industry: facts and figures. Accessed: July 2017.

Ordahl, C.P., E. Berdougo, S.J. Venters, and W.F. Denetclaw. 2001. The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. Development 128: 1731-1744.

Owens, C. M., C. Z. Alvarado. 2015. Market trends and challenges associated with poultry meat quality Proc. Arkansas Nutrition Conference.

Panda, A. K., and G. Cherian. 2014. Role of vitamin E in counteracting oxidative stress in poultry. Poult. Sci. 51:109-117.

Pardue, S. L., J. P. Thaxton, and J. Brake. 1985. Role of ascorbic acid in chicks exposed to high environmental temperature. J. Appl. Physiol. 58:1511-1516.

Peacock, A. J., C. Pickett, K. Morris, and J. T. Reeves. 1990. Spontaneous hypoxaemia and right ventricular hypertrophy in fast growing broiler chickens reared at sea level. Comp. Biochem. Physiol, Part A Mol. Integr. Physiol. 97:537-541.

Petracci, M., S. Mudalal, E. Babini, and C. Cavani. 2014. Effect of white striping on chemical composition and nutritional value of chicken breast meat. Ital. J. Anim. Sci. 13:3138.

Petracci, M., M. Bianchi, S. Mudalal, and C. Cavani. 2013. Functional ingredients for poultry meat products. Trends Food Sci Technol 33:27-39.

Petracci, M., and C. Cavani. 2012. Muscle growth and poultry meat quality issues. Nutrients 4:1.

Pophal, S., P. E. Mozdziak, and S. L. Vieira. 2004. Satellite cell mitotic activity of broilers fed differing levels of lysine. Int. J. Poult. Sci 3:758-763.

Powell, D. J., D. C. McFarland, A. J. Cowieson, W. I. Muir, and S. G. Velleman. 2013. The effect of nutritional status on myogenic satellite cell proliferation and differentiation1. Poult. Sci. 92:2163-2173.

Powers, S. K., J. Duarte, A. N. Kavazis, and E. E. Talbert. 2010. Reactive oxygen species are signaling molecules for skeletal muscle adaptation. Exp. Physiol. 95:1-9.

Puvača, N., and V. Stanaćev. 2011. Selenium in poultry nutrition and its effect on meat quality. Worlds Poultry Sci J 67:479-484.

Qin, N. 2013. The utilization of poultry breast muscle of different quality classes. Helsingfors universitet.

Ruiz-Feria, C. A. 2009. Concurrent supplementation of arginine, vitamin E, and vitamin C improve cardiopulmonary performance in broilers chickens. Poult. Sci. 88:526-535

Russo, E., M. Drigo, C. Longoni, R. Pezzotti, P. Fasoli, and C. Recordati. 2015. Evaluation of white striping prevalence and predisposing factors in broilers at slaughter. Poult. Sci. 94:1843-1848.

Sacheck, J. M., A. Ohtsuka, S. C. McLary, and A. L. Goldberg. 2004. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. Am. J. Physiol. Endocrinol. Metab. 287:E591-E601.

Sahin, K., N. Sahin, and O. Kucuk. 2003. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 C). Nutr Res 23:225-238.

Sanchez Brambila, G., B. C. Bowker, and H. Zhuang. 2016. Comparison of sensory texture attributes of broiler breast fillets with different degrees of white striping. Poult. Sci. 95:2472-2476.

Sandercock, D., R. Hunter, G. Nute, M. Mitchell, and P. Hocking. 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. Poult. Sci. 80:418-425.

Szabó, C. 1996. The pathophysiolgocial role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. Shock 6:79-88.

Schrauzer, G. N. 2000. Selenomethionine: a review of its nutritional significance, metabolism and toxicity. The Journal of nutrition 130:1653-1656.

Shaw, K. 2008. Environmental cues like hypoxia can trigger gene expression and cancer development. Nat Educ, 1, 198.

Sihvo, H.-K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the Pectoralis major muscle of broilers. Vet. Pathol. 51:619-623.

Sobolewska, A., G. Elminowska-Wenda, J. Bogucka, M. Szpinda, K. Walasik, M. Bednarczyk, and M. Paruszewska-Achtel. 2011. Myogenesis–possibilities of its stimulation in chickens. Folia Biol. 59:85-90.

Soglia, F., L. Laghi, L. Canonico, C. Cavani, and M. Petracci. 2016a. Functional property issues in broiler breast meat related to emerging muscle abnormalities. Int Food Res J 89:1071-1076.

Soglia, F., S. Mudalal, E. Babini, M. Di Nunzio, M. Mazzoni, F. Sirri, C. Cavani, and M. Petracci. 2016b. Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. Poult. Sci. 95:651-659.

Solo, J. 2016. Meat quality and sensory analysis of broiler breast fillets with woody breast muscle myopathy. University of Arkansas.

Summers, J. D., D. Spratt, and J. L. Atkinson. 1990. restricted feeding and compensatory growth for broilers. Poult. Sci. 69:1855-1861.

Sumou, I. K., J.-B. Du, B. Wei, C.-Y. Zhang, J.-G. Qi, and C.-S. Tang. 2006. Effect of L-Arginine on pulmonary artery smooth muscle cell apoptosis in rats with hypoxic pulmonary vascular structural remodeling. Acta Biochim. Biophys. Sin. 38:15-21.

Surai, P. F. 2007. Natural antioxidants in poultry nutrition: new developments. Proc. Proceedings of the 16th European symposium on poultry nutrition.

Surai, P. F. 2002. Natural antioxidants in avian nutrition and reproduction. Nottingham University Press Nottingham.

Surai, P. F. 1999. Vitamin E in avian reproduction. Avian Biol Res 10:1.

Swaggerty, C. L., I. Y. Pevzner, H. He, K. J. Genovese, D. J. Nisbet, P. Kaiser, and M. H. Kogut. 2009. Selection of broilers with improved innate immune responsiveness to reduce on-farm infection by foodborne pathogens. Foodborne Pathog. Dis. 6:777-783.

Tan, X., J.-Q. Pan, J.-C. Li, Y.-J. Liu, W.-D. Sun, and X.-L. Wang. 2005. l-Arginine inhibiting pulmonary vascular remodeling is associated with promotion of apoptosis in pulmonary arterioles smooth muscle cells in broilers. Res. Vet. Sci. 79:203-209.

Tamir, H., and S. Ratner. 1963. Enzymes of arginine metabolism in chicks. Arch. Biochem. Biophys. 102:249-258.

Thomas, W., and P. Holt. 1978. Vitamin C and immunity: an assessment of the evidence. J Clin Exp Immunol 32:370.

Tijare, V. V., F. L. Yang, V. A. Kuttappan, C. Z. Alvarado, C. N. Coon, and C. M. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult. Sci. 95:2167-2173.

Trocino, A., A. Piccirillo, M. Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, and G. Xiccato. 2015. Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. Poult. Sci. 94:2996-3004.

Urbani, L., M. Piccoli, C. Franzin, M. Pozzobon, and P. De Coppi. 2012. Hypoxia increases mouse satellite cell clone proliferation maintaining both in vitro and in vivo heterogeneity and myogenic potential. PLoS One 7:e49860

United States Department of Agriculture. Food Safety Inspection Services. 2017. Disposition instructions for “woody breast” and “white striping” poultry conditions. FSIS notice 35-17.

Valceschini, E. 2006. Poultry meat trends and consumer attitudes. In proceedings of the XII European Poultry Conference Verona, Italia.

Van der Slot, A. J., A.-M. Zuurmond, A. F. Bardoel, C. Wijmenga, H. E. Pruijs, D. O. Sillence, J. Brinckmann, D. J. Abraham, C. M. Black, and N. Verzijl. 2003. Identification of PLOD2 as telopeptide lysyl hydroxylase, an important enzyme in fibrosis. Journal of Biological Chemistry 278:40967-40972.

Van Vleet, J., and B. Valentine. 2007. Exertional myopathies. Jubb, Kennedy and Palmer's pathology of domestic animals. 5th ed. Edinburgh: Elsevier:249-254.

Velleman, S. 2007. Muscle development in the embryo and hatchling. Poult. Sci. 86:1050-1054.

Velleman, S. G., and K. E. Nestor. 2003. Effect of selection for growth rate on myosin heavy chain temporal and spatial localization during turkey breast muscle development. Poult. Sci. 82:1373-1377.

Vieira, S. L., and C. R. Angel. 2012. Optimizing broiler performance using different amino acid density diets: What are the limits? J Appl Poult Res 21:149-155.

Vignale, K., J. V. Caldas, J. A. England, N. Boonsinchai, A. Magnuson, E. D. Pollock, S. Dridi, C. M. Owens, and C. N. Coon. 2017. Effect of white striping myopathy on breast muscle (Pectoralis major) protein turnover and gene expression in broilers. Poult. Sci. 96:886-893.

Waldroup, P. W., R. J. Mitchell, J. R. Payne, and K. R. Hazen. 1976. Performance of chicks fed diets formulated to minimize excess levels of essential amino acids. Poult. Sci. 55:243-253.

Wang L. J. Byrem T. M. Zarosley J. Booren A. M. Strasburg G. M. 1999 Skeletal muscle calcium channel ryanodine binding activity in genetically unimproved and commercial turkey populations Poult. Sci. 78 792797.

Whitehead, C., and T. Keller. 2003. An update on ascorbic acid in poultry. Worlds Poult Sci J 59:161-184.

Wideman J. R, R. F., Y. K. Kirby, M. Ismail, W. G. Bottje, R. W. Moore, and R. C. Vardeman. 1995. Supplemental L-arginine attenuates pulmonary hypertension syndrome (ascites) in broilers. Poult. Sci. 74:323-330.

Wilson, P. N., and D. F. Osbourn. 1960. Compensatory growth after undernutrition in mammals and birds. Biological Reviews 35:324-361.

Wood, J., and M. Enser. 1997. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. Br. J. Nutr. 78:S49-S60.

Wu, G. 2010. Functional amino acids in growth, reproduction, and health. Adv Nutr 1:31-37.

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. J Amino Acids 37:1-17.

Wu, G., D. A. Knabe, and S. W. Kim. 2004. Arginine nutrition in neonatal pigs. The Journal of Nutrition 134:2783S-2790S.

Wu, C., T. Dorairajan, and T. Lin. 2000. Effect of ascorbic acid supplementation on the immune response of chickens vaccinated and challenged with infectious bursal disease virus. Vet. Immunol. Immunopathol. 74:145-152.

Xiang, R. P., W. D. Sun, J. Y. Wang, and X. L. Wang. 2002. Effect of Vitamin C on pulmonary hypertension and muscularisation of pulmonary arterioles in broilers. Br. Poult. Sci. 43:705-712.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 20051. Poult. Sci. 93:2970-2982.